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Synthesis Of Apramycin And Paromomycin Derivatives As Potential Next Generation Aminoglycoside Antibiotics And Chemistry Of Isothiocyanato Sialyl Donors

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SYNTHESIS OF APRAMYCIN AND PAROMOMYCIN DERIVATIVES AS POTENTIAL NEXT GENERATION AMINOGLYCOSIDE ANTIBIOTICS AND CHEMISTRY OF ISOTHIOCYANATO SIALYL DONORS

by

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Advisor Date

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DEDICATION

I dedicate my PhD work to my parents, Laxmi and Kesav Reddy who have made many sacrifices in their life and always wish to see me as a better person. I also dedicate my work to my wife Harika Keesara for her endless love and continuous encouragement.

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LIST OF ABBREVATIONS

A	Adenine
Ac	Acetyl
ACN	Acetonitrile
Ada	Adamantyl
ADP	Adenosine diphosphate
AGA	Aminoglycoside antibiotics
AIBN	Azobisisobutyronitrile
AME	Aminoglycoside modifying enzyme
Ar	Aryl
ATP	Adenosine triphosphate
AWMS	Acid washed molecular sieves
BAIB	Bis(acetoxy)iodobenzene
Boc	*tert*-Butyloxycarbonyl
Bn	Benzyl
Bu	Butyl
Bz	Benzoyl
c	Concentration
C	Cytosine
ºC	Celsius
Calcd.	Calculated
Cbz	Benzyloxycarbonyl
CMP	Cytidine-5'-monophospho

m-CPBA	*m*-Chloroperbenzoic acid
DAST	Diethylaminosulfur trifluoride
DCC	*N*,*N*'-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIPEA	Diisopropylethylamine
DMAP	4-(Dimethylamino)-pyridine
DMF	Dimethylformamide
DMP	Dess-Martin Periodinane
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
ESI	Electrospray ionization
EDP	Energy-dependent phase
ESIHRMS	Electrospray ionization high resolution mass spectrometry
Et	Ethyl
Fmoc	9-Fluorenylmethoxycarbonyl
G	Guanine
Gal	Galactose
GalNAc	N-Acetyl galactosamine
gg	*gauche-gauche*
gt	*gauche-trans*
h	Hour
Hz	Hertz
IBX	2-iodoxybenzoic acid

Ipc	Diisopinocampheyl
KDN	Keto deoxy nonulosonic acid
MDR	Multi-drug-resistant
Me	Methyl
mmol	Millimole
mp	Melting point
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	Molecular sieves
NBS	*N*-Bromosuccinamide
Neu5Ac	*N*-Acetylneuraminic acid
Neu5Gc	*N*-Glycolylneuraminic acid
NIS	*N*-Iodosuccinamide
nOe	Nuclear Overhauser effect
PCC	Pyridinium chlorochromate
Ph	Phenyl
Phth	Phthaloyl
PMB	*p*-Methoxybenzyl
ppm	Parts per million
pTSA	4-Toluene sulfonic acid
Py	Pyridine
ROS	Reactive oxygen species
RNA	Ribonucleic acid
Sia	Sialic acid

SFORD	Single frequency off resonance decoupling
TBAF	Tetrabutylammonium fluoride
TBAI	Tetrabutylammonium iodide
TEA	Triethylamine
TEMPO	2,2,6,6-Tetramethylpiperidine-1-oxyl radical
Tf	Trifluoromethanesulfonyl
TFA	Trifluoroacetic acid
TfOH	Trifluoromethanesulfonic acid
tg	*trans-gauche*
THF	Tetrahydrofuran
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
Troc	2,2,2-Trichloroethoxycarbonyl
TTMS	Tris(trimethylsilyl)silane
U	Uracil

CHAPTER 1. AMINOGLYCOSIDE ANTIBIOTICS

1.1. General introduction

The discovery and broad spectrum use of antibiotics may be considered as one of the most important healthcare achievements of the 20th century. The antibiotic era began with the discovery of penicillin by Alexander Fleming in 1928.[1] Since then, a vast number of antibiotics including aminoglycosides, β-lactams, fluoroquinolones and others have been discovered and clinically implemented for treating multiple bacterial infections. Antibiotics can be sorted out into four types based on their mechanism of action: inhibition of cell wall synthesis (most common); inhibition of protein synthesis (second most common class); inhibition of DNA or RNA synthesis; and inhibition of folate coenzyme biosynthesis. The aminoglycosides antibiotics are an essential class of therapeutic agents, which target protein synthesis.[2]

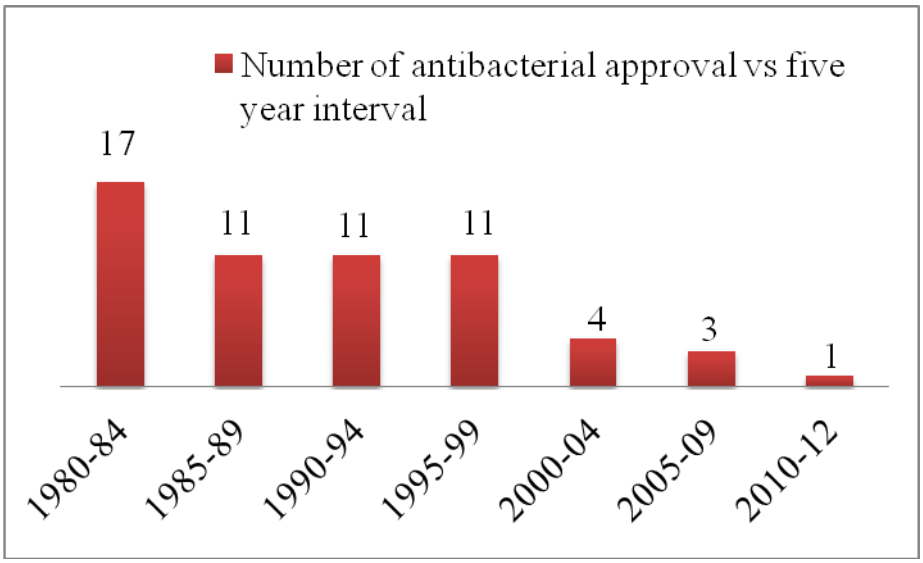


Figure 1: **Number of antibacterial approvals by five year periods (from 1980-2009; 2010-2012 is a 3-year interval)[3]**

AGAs are broad spectrum clinically important antibacterials for human therapy, and have long been used as highly potent antibiotics for treating several bacterial infections. Their efficacy is demonstrated against many Gram-negative and Gram-positive pathogens, as well as against

multi-drug-resistant tuberculosis, strains of MRSA, and against complex infectious diseases such as exacerbated cystic fibrosis. Part of the reason why AGAs suffer from severe resistance problems is because of their extreme use in the healthcare system. Many bacterial strains have become resistant to regular doses of aminoglycosides through the evolution and action of AMEs.[4] The consequence of antimicrobial resistance to AGAs on the human curative process, has been recognized by the WHO, which released a global action plan to address this menace.[5] The morbidity rate due to antibiotic resistant-infections in the USA is two million people/year, with at least 23,000 dying as a result, with estimates of $20 billion in excess direct healthcare costs.[3] If drug-resistant infections are not tackled well, they could result in 50 million deaths and $100 trillion of treatment costs by 2050, according to an estimate by an official commission in UK.[6] Further, the clinical utilization of AGAs and their development into therapeutics is limited because of their toxicity, in particular, the irreversible hearing damage called ototoxicity, and reversible kidney damage called nephrotoxicity. In addition, the number of new antibiotics approved by the FDA, has gradually decreased in the last three decades, which minimizes the options to treat bacterial resistant infections (Figure 1). Attempts aiming to generate better aminoglycosides have employed several approaches including chemoenzymatic modification and coupling of antibiotics. Toward this end aminoglycosides, like most other classes of drugs, have been extensively modified by synthetic or semisynthetic routes.[4]

The first of this thesis is directed towards developing new aminoglycoside antibiotics with emphasis on their chemical synthesis, and the biological evaluation of newly synthesized analogues, as well as the exploration of structure-activity relationships to obtain knowledge about antimicrobial activity. The goal of the research was the design and development of more active and less toxic aminoglycoside antibiotics. In particular, studies have focused on the

modification of the aminoglycosides, apramycin and paromomycin so as to develop the next generation of potent AGAs.

1.2. History and structural features of AGAs

Streptomycin, the first aminoglycoside antibiotic drug discovered in the 1940s, was isolated from *Streptomyces griseus*, used successfully to treat tuberculosis, and has been in use as an antibiotics for over 60 years. After successful entry of streptomycin into clinical treatment against microbial infections, several other novel AGAs have followed to fight against bacterial infections. Some notable active AGAs are shown in Table 1. Most of the AGAs are available as natural compounds, which are obtained from actinomycetes of either genus *Micromonospora* (indicated as-"micin") or genus *Streptomyces* (indicated as -"mycin").

Table 1: **Sources and year of discovery of some notable AGAs**

S.No	Aminoglycoside antibiotic	Source	Year
1	Streptomycin	*Streptomyces griseus*	1944
2	Neomycin	*Streptomyces fradiae*	1949
3	Kanamycin	*Streptomyces kanamyceticus*	1957
4	Paromomycin	*Streptomyces rimosus forma paromomycinus*	1956
5	Gentamicin	*Micromonospora purpurea*	1963
6	Tobramycin	*Streptomyces tenebrarius*	1967
7	Apramycin	*Streptomyces tenebraius*	1968
8	Sisomicin	Genus *Micromonospora*	1970
9	Butirosin	*Bacillus circulans*	1971

10	Dibekacin	Semisynthetic derivative of Kanamycin B	1971
11	Amikacin	Semisynthetic derivative of Kanamycin B	1972
12	Arbekacin	Semisynthetic derivative of dibekacin	1973
13	Isepamicin	Semisynthetic derivative of Gentamicin B	
14	Netilmicin	1-N-ethyl sisomicin	1976

As with other classes of antibiotics, resistance and toxicological issues observed in clinical usage of AGAs have become widespread. This has led to efforts to develop the pharmacological profile of AGA's and the consequent introduction of a second generation of AGAs, also known as semi-synthetic derivatives, as shown in the Table 1. However, toward the end of 1970s, the introduction of a broad range of other antibiotics with lower side effects diminished the interest in the search for new AGAs. Isepamicin (1988) and arbekacin (1990) were the latest approved AGAs to be approved.[7] However, this situation may change as plazomicin, a semi-synthetic AGA derived from sisomicin developed by Achaogen, recently completed phase II clinical trials for urinary tract infections.[8]

Despite the reduced interest the use of AGAs in the clinic continues, in part because of the growing resistance to all other general antibiotics. AGAs are currently predominantly used in nosocomial infections and in particular for severe Gram-negative infections. AGAs are water-soluble, polycationic pseudo oligosaccharides with several hydroxyl and amino groups. Their structure consists of several aminated sugars connected by glycosidic linkages to a dibasic cyclitol. The AGAs are heavily protonated under physiological conditions, and have strong

affinity toward negatively charged nucleotides. The pK_a values of individual amino groups of AGAs range from 5.7 to 10.1.[9,10] AGAs have a relative low molecular weight (500 to 800) and are most frequently isolated as sulfate and acetate salts. Most are odorless, and white to off-white amorphous powders.

1.3. Structural classification

AGAs, also known as aminoglycoside-aminocyclitol antibiotics, act as therapeutic agents by inhibiting protein synthesis. The term ʻaminoglycosideʼ generally refers to any carbohydrate that carries an amino functionality. The common aminoglycoside structural motif consists of a cyclitol derivative linked to minimum of one aminosugar, with the complete structure containing at least two amino functionalities and a number of free hydroxyl groups, which may also have further substituents.

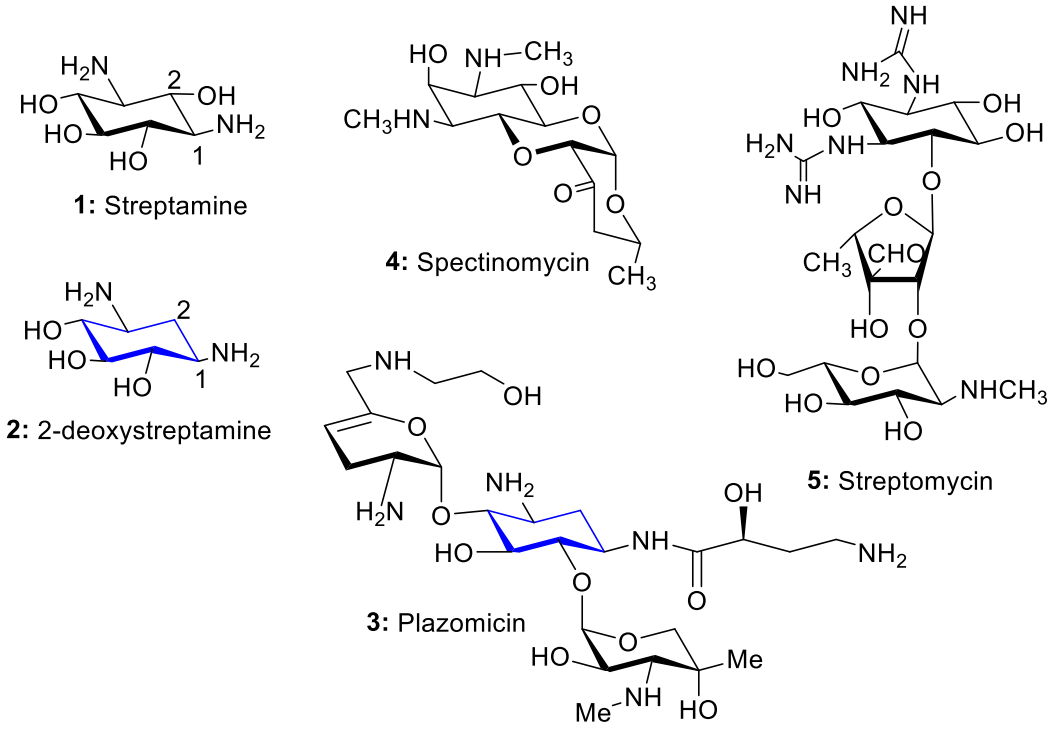


Figure 2: **Structure of streptamine (1), 2-deoxystreptamine (2), Plazomicin (3), and examples of AGAs, that are not derived from 2-deoxystreptamine (4, 5)**

Most aminoglycoside molecules contain a central aminocyclitol ring, 2-deoxystreptamine linked to one or more amino sugars by pseudo glycosidic bonds. 2-Deoxystreptamine plays a vital role in aminoglycoside biological activity. The first AGA, streptomycin (5, Figure 2), belongs to a relatively rare class of AGAs that is made up of a scaffold of the disaccharide unit linked to the 4-position of a guanidinylated streptamine.

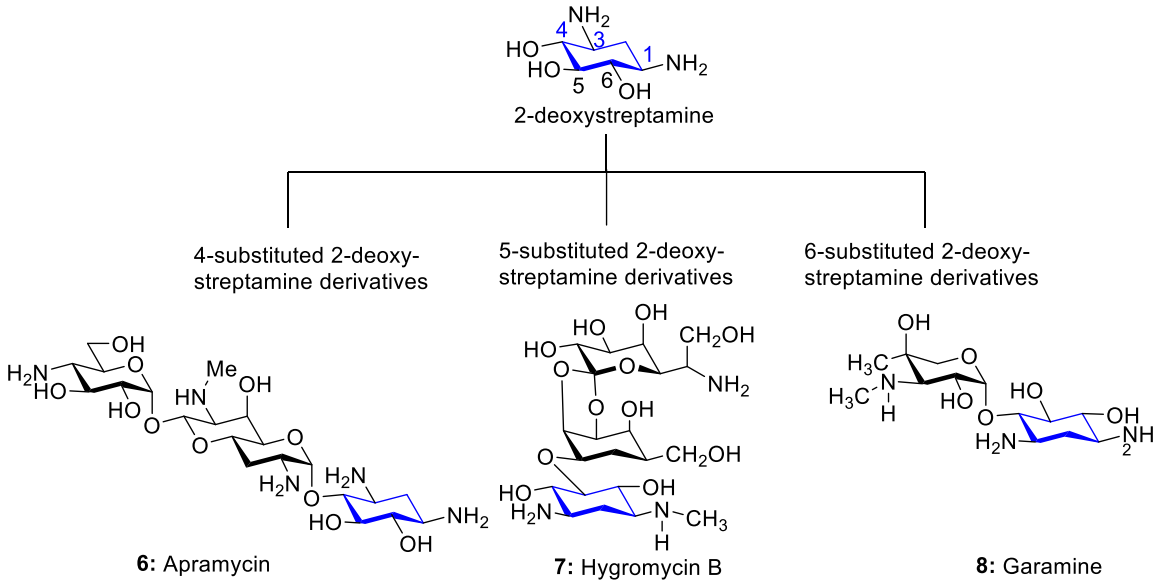


Figure 3: **Classification of monosubstituted 2-deoxystreptamine derivatives**

Most AGAs can be sorted into two major classes: a large number of compounds containing 2-deoxystreptamine derivatives, and compounds without the 2-deoxystreptamine motif (*e.g.*, streptomycin (**4**) and spectinomycin (**5**)) (Figure 2). The group containing the core scaffold 2-deoxystreptamine is the most essential and is usually further classified into mono and disubstituted 2-deoxystreptamine derivatives as shown in (Figures 3 & 4).

A large number of AGAs contain 2-deoxystreptamine as a center scaffold, and are biosynthetically derived from paromamine. These are classified into 3 major classes based on substituents of the core paromamine moiety, namely kanamycins, neomycins, and gentamicins.

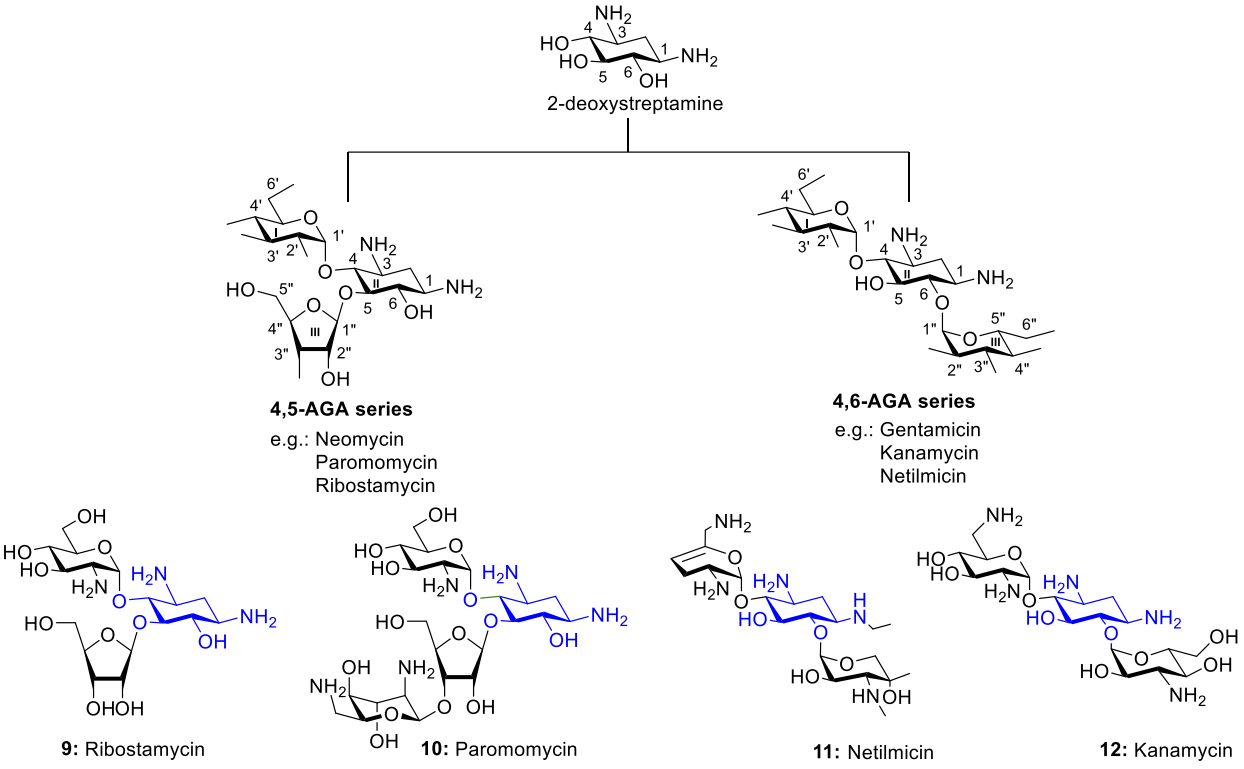


Figure 4: **Classification of disubstituted 2-deoxystreptamine derivatives**

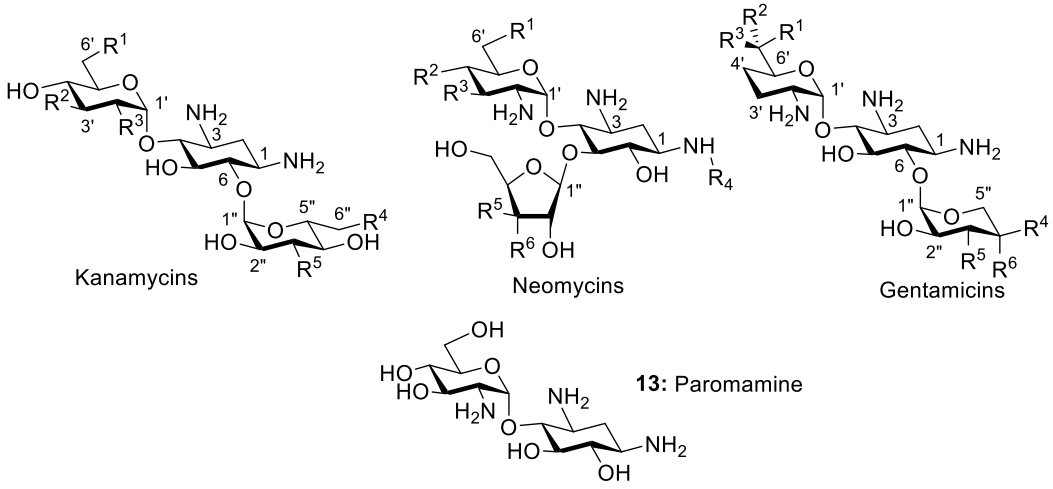


Figure 5: **Structures of the AGA classes based on paromanine: kanamycins, neomycins, and gentamicins**

The majority of clinically applied AGAs belong to the paromamine-derived AGAs. Of these the kanamycin family consists of 4,6-disubstituted-2-dexoystreptamine analogues with both a 2-amino or 2,6-diamino-glucose ring at the 4-position and a 3-amino substituted glucose ring at the 6-position. The neomycin category are of 4,5-disubstitued 2-dexoystreptamines with one furanoside and one or two pyranosides. Finally, the gentamicin class are 4,6-disubstituted 2-dexoystreptamines, usually with two hexose rings and an additional carbon side chain (Figure 5).[7]

1.4. Mode of action of AGAs

1.4.1. Functional and structural features of the ribosome

Since the discovery of streptomycin in 1943, aminoglycosides have served as chemotherapeutic agents in the treatment of a variety of bacterial infections, whose efficacy has been proven against a number of clinical pathogens, including both Gram-positive and Gram-negative pathogens.[11] Although the detailed mechanism of action of AGAs is still under investigation, AGAs mainly target the bacterial ribosome 16S decoding A site by direct interaction with ribosomal RNA thereby affecting bacterial protein synthesis by inducing codon misreading or by inhibiting translocation of the tRNA-mRNA complex.[12]

DNA and RNA are central players in gene expression, transmission and conservation of the genetic information. DNA directs its own replication (conservation) and through transcription (transmission) yields RNA, which in turn through translation (gene expression), leads to protein synthesis. This information flow is known as the central dogma of molecular biology.[13] RNAs are constructed with four main nucleotides, each one of which has three constituents: a nucleobase, a ribofuranose, and a phosphodiester group. Among the four nucleobases, two are

purines; adenine (A) and guanine (G), and the remaining two are pyrimidines; uracil (U) and cytosine (C) (Figure 6).

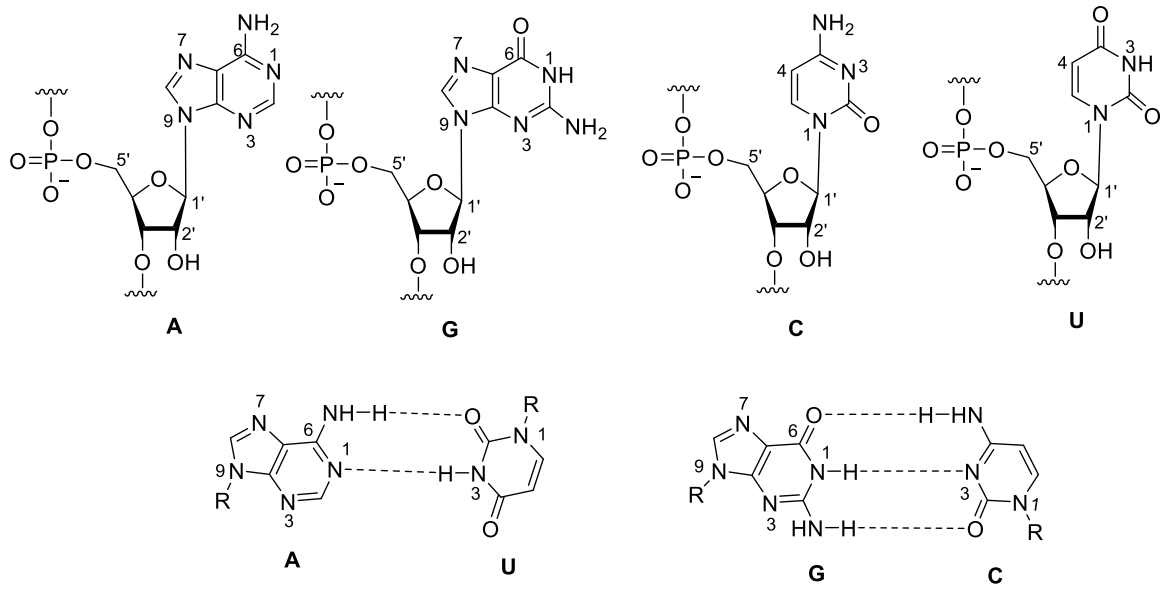


Figure 6: **The four fundamental RNA nucleotides and the Watson-Crick base pairs**

A codon is a part of mRNA, consisting of three consecutive nucleotides, whose sequence indicates one of the 20 amino acids. Each tRNA has an anticodon that matches with a specific mRNA codon. In the ribosome, codons on the mRNA travels through two channels on the small subunit to attain the decoding site where the codon contacts with the tRNA anticodon. Peptide bond formation is initiated when the first codon on the mRNA interacts with the initiator tRNA containing the amino acid methionine at the peptidyl site (P-site). Such peptide bond formation is illustrated in Figure 7. Protein synthesis by mRNA translation in the ribosome must be rapid and accurate. This accuracy, is achieved by the perfect pairing of three bases between the mRNA codon and the tRNA anticodon. The anticodon should have a perfect match with the first two codon positions based on the Watson-Crick base-pairing rules (Figure 6).[14-18]

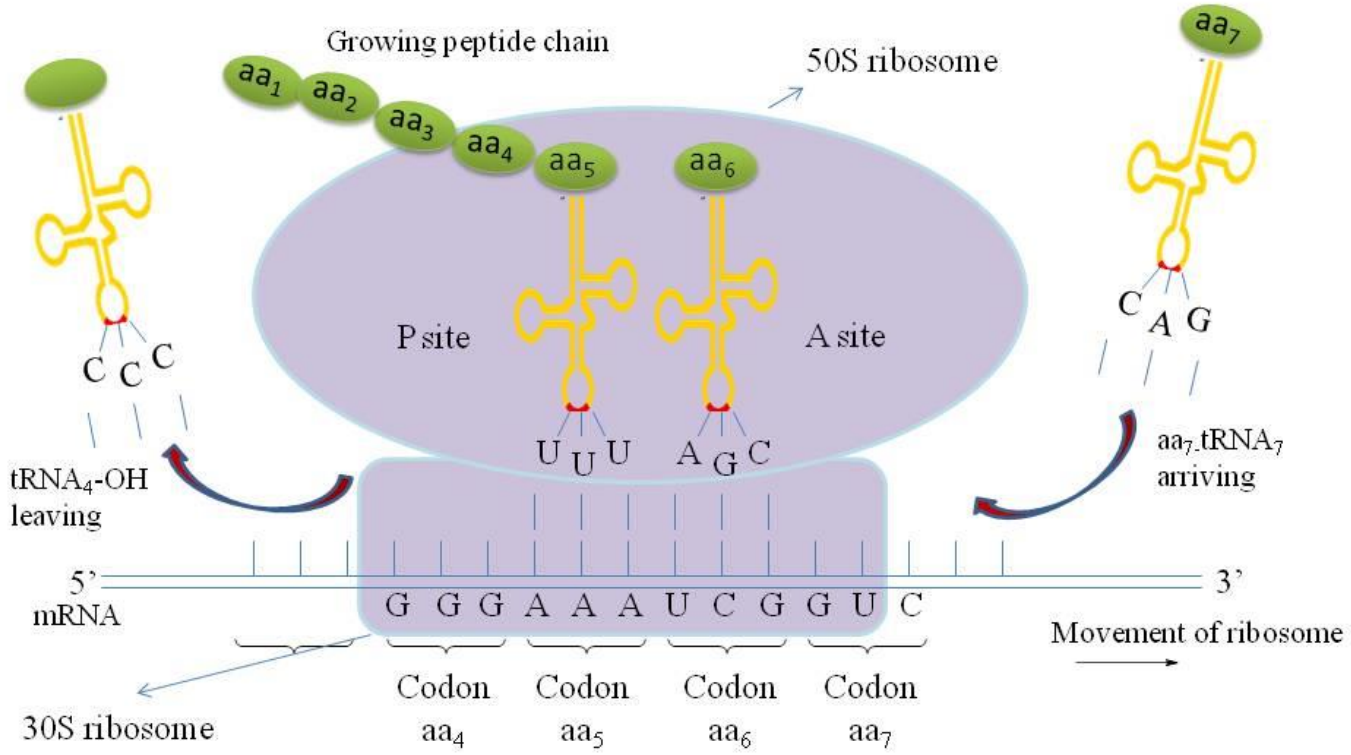


Figure 7: **Schematic representation of peptide bond formation**

1.4.2. Uptake and mode of action

Because the AGAs are water-soluble, polycationic oligosaccharides with several hydroxyl and ammonium groups they are heavily protonated under physiological conditions, and have strong affinity toward negatively charged nucleotides. There are two types of interactions that support the recognition and binding of AGAs to their bacterial rRNA targets. The most significant contribution is from electrostatic interactions and these are supplemented by hydrogen bonding between multiple amino and hydroxyl functionalities of the AGAs and the RNA bases. The antimicrobial activity of AGAs is an outcome of a multi-step process. The first

step requires the AGAs to reach their molecular target, for which they must penetrate into the cytoplasm of the bacterial cell. The mechanism by which AGAs penetrate into Gram-negative bacteria remains ambiguous. Nevertheless, according to the currently accepted mechanism, it consists of three consecutive steps. AGA uptake is the first stage, and is simply an electrostatic interaction between the positively charged aminoglycosides and the negatively charged lipopolysaccharides of the surface bacterial membrane.[19,20] This interaction is mostly nonspecific and is solely due to the cationic environment of the AGAs resulting from the basic, ionizable amino groups. The subsequent stages are energy dependent steps known as EDP-I and EDP-II. After entering into the periplasmic space, the AGAs are transported through the cytoplasmic (inner) membrane. This step, which depends on electron transport and is the rate-determining step (slow rate), is termed as EDP-I and is associated with AGA concentration. Subsequently, the AGAs bind to the 30S ribosomal subunit, which is the EDP-II.[21] AGAs interfere with protein synthesis (translation process) upon binding to rRNA, leading to membrane damage, which leads to the accumulation of the antibiotic in the cell, and eventually to cell mortality.[22]

The influence of AGAs on bacterial protein synthesis was first revealed in 1965.[23] AGAs mainly target the bacterial ribosome by direct interaction with ribosomal RNA thereby affecting protein synthesis by inducing codon misreading or by inhibiting translocation of the tRNA-mRNA complex.

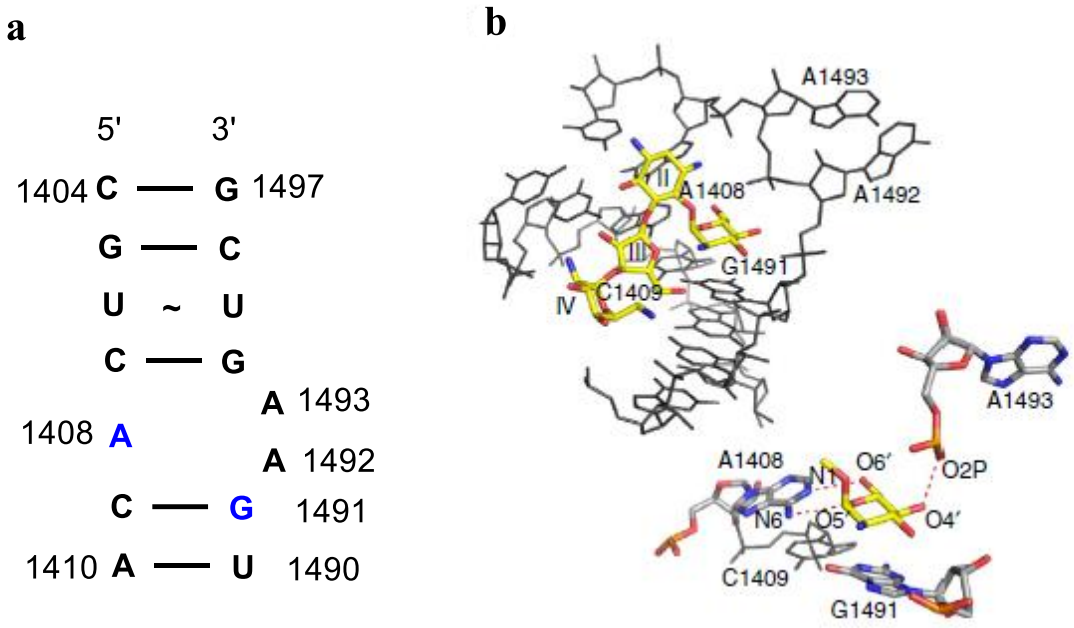


Figure 8: **(a) Secondary structure of the AGA-binding pocket in helix 44 of 16S rRNA in complex with paromomycin. Key residues for selectivity of AGAs are 1408 and 1491 (blue). (b) Detail of the paromomycin ring 1 bound to the bacterial A site[24]**

The AGA molecular target is on helix 44 in the 16S rRNA subunit; the precise binding location is part of the aminoacyl-tRNA acceptor site, which is known as the decoding A site.[25] Although different modes of binding have been observed with a variety of aminoglycoside derivatives, the general interaction of AGAs with three unpaired adenine residues in the decoding loop displace the non-complementary adenines (A1492 and A1493) and locks them into a so-called "flipped-out" orientation (Figure 8).[4] While majority of AGAs efficiently inhibit the propagation level in protein translation, their approaches are mechanistically varied. Thus, AGAs influence bacterial protein synthesis either by hindering translocation of the tRNA-mRNA complex or by provoke misreading.[25]

The two rings of the neamine core (rings I and II) are common for the 4,5- and 4,6-classes of AGAs, and are the main contributor to binding to the rRNA. They interact through

hydrogen bonding of the 6'- substituent (OH in paromomycin, NH_2 in neomycin) and the ring oxygen with N1 and N6 of the highly conserved A1408 of helix-44 in the narrow drug binding pocket. The 2-deoxystreptamine (ring II) of the neamine core forms hydrogen bonds to U1406, U1495 and G1494 of the binding site. Based on the substitution pattern of the AGA, ring I binds to a number of ribosomal bases including A1408, A1493, A1492, and G1491.[24] There are no significant binding interactions of ring III and ribosomal RNA. The additional rings attached to the deoxy streptamine ring at the 5- or 6- positions, might have impact have an AGA's specificity. Additional hydrogen bonding interactions form between various hydroxyl groups of the neamine core and the phosphate groups linked to the ribonucleosides in the binding pocket through water mediation. Paromomycin and apramycin represent two diverse mechanisms of action.

Paromomycin binds to the 16S bacterial RNA binding site in the major groove of helix 44. The glucopyranosyl (ring I) of paromomycin, forms hydrogen bonds with A1408, A1492, A1493, and the base pair C1409–G1491 (Figure 8). Ring II forms strong hydrogen bonds with 16S bacterial RNA, in particular, the phosphate backbone of A1493; such is the strength that it locks the skeleton in the ''flipped-out'' form. This leads to reduced binding affinity of cognate and non-cognate tRNA codons and as a result codon misreading takes place.[12] Paromomycin ring I behaves like a nucleotide base, hydrogen-bonding with A1408 (C(6')-OH to N(1) of A1408) and stacking on top of the purine ring of G1491 (Figure 8).

All studies so far have concluded that paromomycin, and most of the 2-deoxystreptamine derived AGAs influence the fidelity of translation of the ribosome by enhancing the incorporation of near-cognate tRNAs. Most 4,5- and 4,6-AGAs bind to the decoding A site in a similar manner to paromomycin as revealed by various X-ray structures, and lead to a similar

loss of translational accuracy.[26] The details of how this misreading leads to cell death are not well understood, as for example ribosomal mutants with reduced translational accuracy are still feasible.[27] It has been suggested that AGAs enhance the permeability of cell membranes as a result of the incorporation of inaccurate proteins, with the consequence of subsequent saturation of the ribosome with AGAs and most likely complete inhibition of protein synthesis.

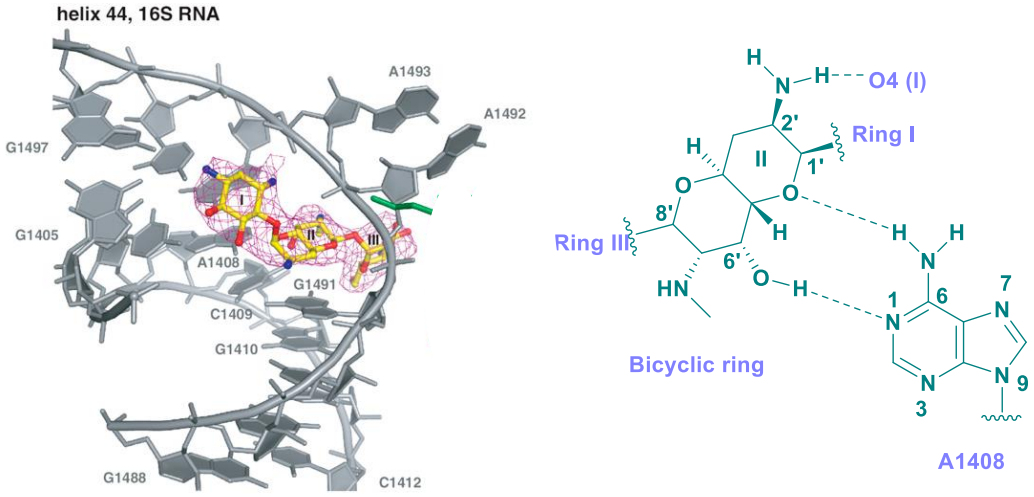


Figure 9: **Decoding site of 30S subunit (*Thermus thermophilus*) in complex with apramycin and pseudo base pair interaction of the bicyclic sugar (II) of apramycin with the A1408 residue**

X-ray crystallographic study with the absolutely constituted bacterial ribosome reveals that the apramycin bicyclic ring II is bind to the ‘‘flipped-out’’ conformation of the bacterial ribosomal A site similar way to ring I of the 4,5- and 4,6-AGAs. In particular, the β-face of ring II (bicyclic ring) interacts through CH-π interactions with the bacterial ribosomal base G1491; the 6'-OH group serves as hydrogen bond donor with *N*1 of the ribosomal base A1408 and another ring oxygen O1' acts as hydrogen bond acceptor with amine group of A1408 (Figure 9).[26] The hydrogen-bonding pattern and location of the apramycin 2-deoxystreptamine ring are analogous to those of the 2-deoxystreptamine moiety in the decoding A-site complexes of the 4,6-disubstituted AGAs. The key hydrogen bonds between apramycin *N*3 and *N*7 of G1494;

apramycin *N*1 and *O*4 of U1495.[25,28] The terminal sugar (4-amino-4-deoxy-D-glucose) of apramycin forms hydrogen bonds with base pair C1409 and G1491. In addition, 2'''-hydroxy group also form hydrogen bonds with G1491 and A1492.[28]

On the other hand, crystallographic studies of apramycin bound to the model sequences, which corresponds to the eukaryotic ribosomal A site disclose that the drug binds to a ‘‘flipped in’’ conformation in which A1492 forms an hydrogen bond to G1408. In this complex bicyclic ring II of apramycin is rotated with respect to its orientation in the bacterial complex and does not stack with A1491 and 2'-NH_2 group of apramycin forms a hydrogen bond to the G1408 O6 leaving the apramycin 6'-OH exposed to water.[29,30]

In contrast to the other AGAs, apramycin acts initially by blocking the translocation of ribosome along mRNA, and as a result, gives only limited codon misreading. One reason could be the hydrogen bond interaction between 2''' hydroxyl group of terminal ring III and the ribose moiety of A1492, which possibly involve in the switch of nucleotide A1492 to adopt a ‘‘flipped-out’’ conformation as is connected with aminoglycoside-induced misreading. It is consistent with the aprosamine (lack of terminal ring), readily induced misreading on bacterial ribosomes.[25,28]

1.5. Problems associated with therapeutic usage of AGA's

The clinical use of AGAs and their applications into therapeutics is limited because of three major problems. The first and foremost problem is associated with two types of toxicity; reversible nephrotoxicity and irreversible ototoxicity. In spite of the therapeutic effects of AGAs, their use requires careful monitoring of patients as both toxicities are dose-dependent. The second problem, as with all antibiotics, is the rise of resistance as a result of overuse and misuse. Numerous resistance mechanisms have been elucidated.[7] The third problem is the complexity

associated with the total and partial syntheses of AGAs. Since the beginning of the AGAs era, these problems have limited their therapeutic usage and further development.

1.5.1. Toxicity of aminoglycoside antibiotics

A number of toxicities have been identified with AGAs in medical use, including ototoxicity[31] (vestibular and auditory), nephrotoxicity[32], retinal toxicity, and, infrequently neuromuscular blockage. Among these, ototoxicity and nephrotoxicity are of primary concern. Inherent toxicity allied with nonspecific binding to RNA a further problem that obstructs wider adoption of AGAs.

1.5.1.1. Nephrotoxicity

Kidney damage is called nephrotoxicity. Drug induced nephrotoxicity leads to the body's incapability to eliminate urine and other wastes. If not treated well, it leads to an eventual concomitant growth of electrolytes in the blood and consequently permanent kidney breakdown. Primarily, AGAs are eliminated by glomerular filtration and excretion in the urine and, consequently, accumulation of AGA is observed in the kidneys. Accumulation of about 5% of the administrated dose of an AGA in the epithelial cells (one of the four fundamental types of animal tissue) of the proximal tubules can be lead to the nephrotoxicity. Significant accumulation of an AGA in the renal cortex (outer portion of kidney) tissue is a strong indication of aminoglycoside-induced nephrotoxicity.[32] Nephrotoxicity is minimized in the clinic by administration of a single large daily dose rather than several smaller doses.[33] Netilmicin is the reportedly the least toxic, whereas gentamicin is considered the most toxic. Amikacin and tobramycin are the best tolerated AGAs.[34-36]

1.5.1.2. Ototoxicity

Another important obstruction to AGA clinical therapy is ototoxicty, which is mostly irreversible and affects ~20% of the patient population.[37] Ototoxicity is connected with the destruction of the sensory cells of the inner ear. Two types of ototoxicity are presented; cochlear toxicity and vestibular toxicity. All AGAs exhibit ototoxicity but differ in toxic potential and organ preference, i.e., preferential damage to the cochlea or vestibule.[38] Neomycin is believed to be highly toxic while gentamicin, tobramycin, and the kanamycins exhibit moderate toxicity. Neomycin and amikacin influence mostly the cochlea whereas gentamicin is considered to be vestibulo toxic. That AGAs cause ototoxicity can be explained by two mechanisms. In the first mechanism, the formation of reactive oxygen species (ROS) is believed to be the initiation step that is followed by further events that ultimately lead to cell death. Aminoglycoside antibiotics can form complexes with iron present in cells to activate dioxygen, and form the superoxide radical (a ROS) and lipid peroxides by reaction with polyunsaturated fatty acids such as arachidonic acid. Lipid peroxides can commence a chain reaction of peroxidation (radical degradation), and reactive oxygen species can undergo a Fenton-type reaction to form hydroxyl radicals. Together these reactions, Fe(II) catalysed reduction of molecular oxygen and an subsequent chain reactions with formation of different ROS, result in damage to the cell (Figure 10).[39,40]

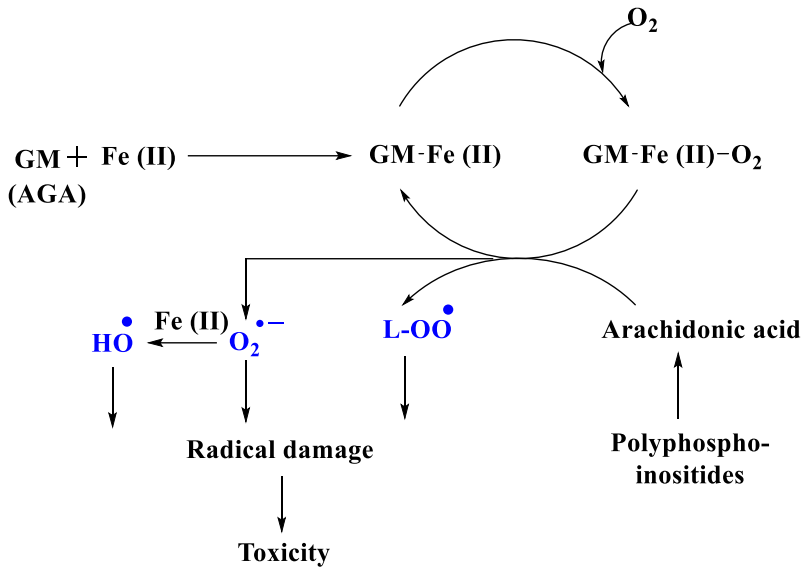


Figure 10: **Proposed mechanisms for aminoglycoside-induced ototoxicity**

Further, Böttger and co-workers proposed, on the basis of genetic studies of aminoglycoside interactions with eukaryotic ribosomal RNA, that AGAs inhibit mitochondrial protein synthesis which enhances the cochlear toxicity associated with aminoglycosides.[41] Crystallographic analyses of rRNA hybrids of human wild-type, the human A1555G mutant, and bacterial decoding A-sites, strongly support the hypothesis that AGA induced deafness is affected by genetic factors. Ototoxicity occurs in a couple of ways. i) a random dose dependent manner in the common patient population, and ii) in an aggravated type in genetically susceptible individuals, with the latter linked to mutations in mitochondrial rRNA, in particular, the transition mutations A1555G and C1494U in the A-site of the mitochondrial ribosomal RNA subunit.[41,42] The sequence differences between the rRNA subunit decoding A sites of the eukaryotic and prokaryotic ribosomes are minimum. As a result, competitive AGA binding to the human ribosome is expected (Figure 11).

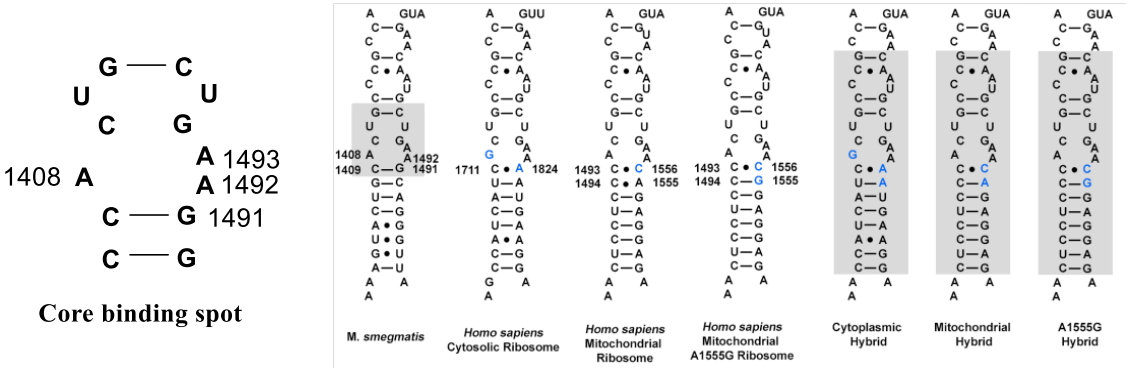


Figure 11: **Core binding region and secondary-structure comparison of prokaryotic and eukaryotic ribosomal decoding site rRNA sequences in the small ribosomal subunit[25]**

The investigation of interactions between bacterial ribosome and AGAs is not only important to understand the mode of action of aminoglycoside, but also to probe the structure and function of the ribosomal decoding site. Several methods were developed to study the complexes of AGAs and the decoding A site including NMR structural studies of AGAs complexed with A site models.[43,44] Relatively well resolved crystal structures of the 30S ribosomal subunit, in the presence and absence AGAs led to a major breakthrough in understanding their mechanisms of action.[12,45,46] Besides NMR and X-Ray crystallography techniques, several other methods were developed to look into the interactions between AGAs and the ribosomal A site.

The ribosomal drug susceptibility is studied by *in vivo* measurement of the minimal inhibitory concentration against a single rRNA allelic derivative of the *Gram-positive eubacerium Mycobacerium smegmatis*.[47] MIC is defined as the lowest concentration of an antibiotic substance that absolutely inhibits the visible growth of a microorganism after overnight incubation.[48] To determine the susceptibility of microorganisms to antimicrobial agents, MICs are considered as the ‘golden standard’. Böttger *et al*. established cell-free translation assays with purified 70S ribosomes of both wild-type and mutant M. smegmatis strains to evaluate the effect

of AGAs on the fidelity of translation.[14] For that, they constructed a large number manipulated mutants with defined alterations in the ribosomal A site (Figure 12 A).

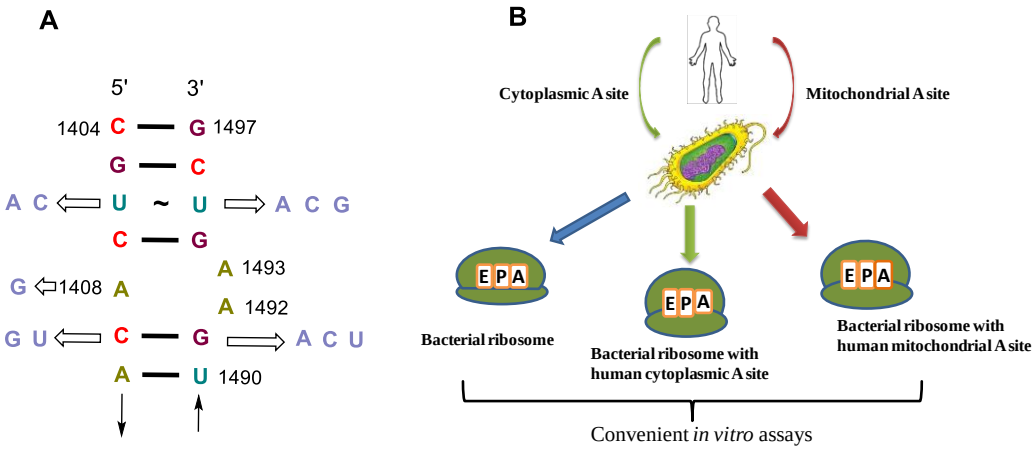


Figure 12: **A) Secondary structure of the bacterial A site (mutations introduced are shown in blue), B) Bacterial hybrid ribosome with a fully functional eukaryotic rRNA decoding site**

The incorporation of the complete decoding A site cassette of human mitochondrial (wild-type and recombinant mutants) and cytosolic rRNA into bacterial rRNA has facilitated the development of cell-free translation assays, to investigate the AGAs inhibition of mitochondrial, cytosolic, and bacterial protein synthesis.[49] These assays envisage both AGA antibacterial activity as well as drug selectivity at the target level, which leads to the development of potent AGAs (Figure 12B). To estimate the antibacterial activity of the aminoglycosides on protein synthesis (translation), IC_{50} values were determined. The IC_{50} value is the concentration of substance (AGA) required to inhibit the bacterial protein synthesis. In particular, antibacterial activity is measured against clinical isolates of *Escherichia coli* (3 strains) and methicillin-resistant *Staphylococcus aureus* (4 strains), which were isolated from patients.

1.5.2. Resistance

AGAs also suffer from severe resistance problems because of their wide-ranging use against human and animal pathogens. Both the extended term exposure to low dosage of AGAs and the failure to complete a prescription promote more resistant bacterial strains. So far, three bacterial resistance mechanisms to AGAs have been identified. The first mechanism involves reduction of the intracellular concentration of AGA either by limiting drug uptake or by enhancing the activity of active efflux systems. The second mechanism is the alteration of the target 16S RNA bacterial ribosomal subunit. Finally, the last and the most important resistance mechanism is the enzymatic modification of AGAs or deactivation of AGAs (Figure 13).[4,50] With respect to the latter three classes of aminoglycoside modifying enzymes (AME) are majorly affecting the AGAs. They are the aminoglycoside phosphotransferases (APHs), the aminoglycoside acetyltransferases (AACs), and the aminoglycoside nucleotidyltransferases (ANTs).

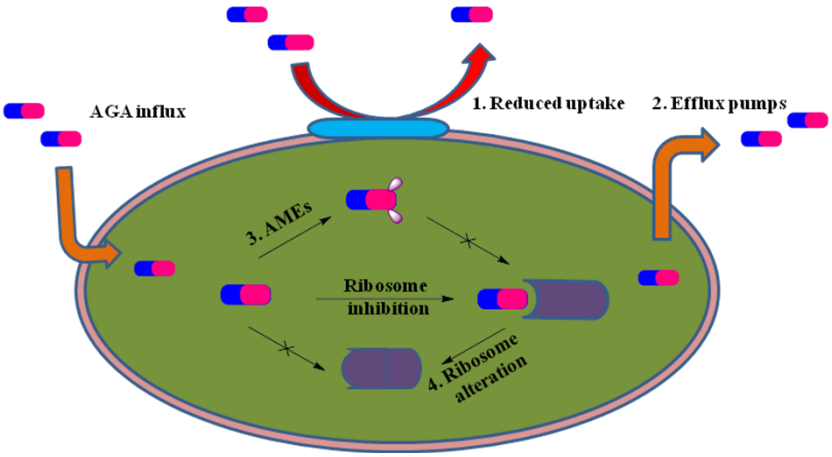


Figure 13: **Schematic outline of mechanisms of resistance to AGAs**

1.5.2.1. Reduced uptake and increased efflux

The decline of drug uptake and/or activation of drug efflux leads to a reduction in the AGA concentration in target bacterial cells.[51] This mechanism influences the susceptibility of the strain to the entire family of aminoglycoside antibiotics and is a source of intrinsic resistance. Even though the exact mechanism of aminoglycoside uptake is still unclear (section 1.4.2), it is thought that the process consists of three subsequent steps. The first step is the adsorption of the polycationic AGA to the surface of bacteria by electrostatic interactions with the negatively charged lipopolysaccharides found on the outer cell membrane of Gram-negative bacteria. The two subsequent steps are oxygen dependent, and thus anaerobic bacteria are intrinsically resistant to aminoglycosides.[52] Energy-dependent bacterial efflux pumps have now been identified as a main source of resistance to antibiotics, in particular, in the case of multidrug-resistant pathogens accountable for nosocomial infections. Multidrug efflux pumps are active transporters and are made up of proteins that are localized in the cytoplasmic membrane of all types of cells. They need a source of chemical energy to carry out their function. A few primary active transporters use adenosine triphosphate hydrolysis as a source of energy.[51]

1.5.2.2. Modification of the target RNA

In this mechanism of resistance, the aminoglyocisdes target, the .16S RNA sub unit of the bacterial ribosome (Figure 13) is altered by a bacterial modification. Members of the actinomycetes group generate inactive aminoglycosides such as partially phosphorylated or acetylated ones, which are cleaved during or after their export out of the cell by particular enzymes to provide the active antibiotics compounds.[51] *Streptomyces spp.* and *Micromonospora spp.*, a class of aminoglycoside-producing organisms are capable of expressing rRNA methylases, which can methylate the 16S rRNA at particular positions that are crucial for the

binding of the drug.[53] Several rRNA methylases have been studied.[54] For example, KamA isolated from *Streptomyces tenjimariensis* and KamB isolated from *Streptomyces tenebrarius* are methylation genes; their gene products catalyze the modification of N(1) of A1408 leading to elevated resistance to most of the AGAs including kanamycin, sisomicin, tobramycin, and apramycin, but not to gentamicin.[55] Methylation of A1408 results in the loss of contact with the 6'-group of the aminoglycosides which is thought to be critical for antibiotic activity.

1.5.2.3. Enzymatic Modification of the aminoglycoside

The enzymatic modification of amino or hydroxy groups of AGAs by specific enzymes, is the main cause of aminoglycoside resistance in clinical isolates of Gram-negative and Gram-positive bacteria. The modified AGAs bind weakly to the target ribosome, resulting in the loss of antibacterial activity[56] (Figure 14). Three kinds of aminoglycoside-modifying enzymes (AMEs) are have been identified:

• Aminoglycoside Acetyltransferases (AAC)

• Aminoglycoside Phosphotransferases (APH)

• Aminoglycoside Nucleotidyltransferases (ANT)

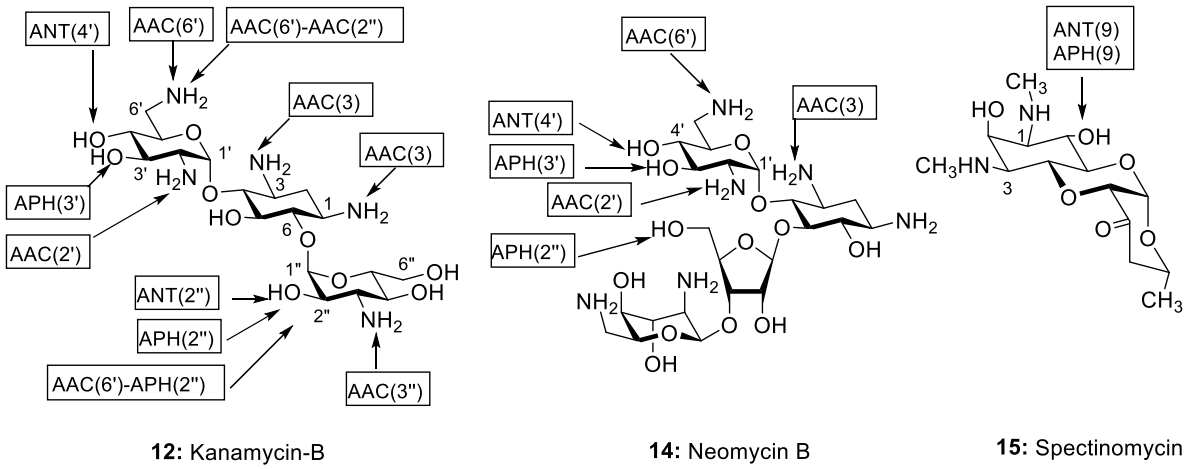


Figure 14: **Main stream aminoglycoside-modifying enzymes and their effect on kanamycin B (a 4,6-2-deoxystreptamine derivative), neomycin B (a 4,5-2-deoxystreptamine derivative) and spectinomycin**

1.5.2.3.1. Aminoglycoside Acetyltransferases (AAC)

AACs are considered as a main source of resistance in Gram-negative organisms (*Enterobacteriacae*), and are also detected in Gram-positive pathogens (*Staphylococci, Enterococci*).[57] Around 50 members of the AAC family have been recognized. Among them, four major classes have been identified; AAC(1), AAC(3), AAC(2'), and AAC(6'). These enzymes catalyze the regioselective N-acetylation of an amino group of the aminoglycoside utilizing acetyl-CoA as a donor. They can alter the 1- and 3-amino groups of the central 2-deoxystreptamine ring (ring II) and the 2'- and 6'-amino groups of the 6-deoxy-6-aminoglucose ring (ring I) Figure 14.

The 6'-amino group of the aminoglycosides plays a vital role in rRNA binding to the 30S ribosomal subunit. Thus, the 6'-position is the target of one of the numerous classes of aminoglycoside- modifying enzymes. AAC(6') enzymes are capable of modifying the majority of the clinically significant AGAs. This subclass contains more than 25 members. AAC(6') type-1 of is cause for resistance to the many useful AGAs.[4,7]

1.5.2.3.2. Aminoglycoside Phosphotransferases (APH)

O-Phosphorylation of hydroxyl groups in AGAs by APH enzymes is usually observed in Gram-positive bacteria such as *S. aureus*. As a result of APH action a negative charge is introduced into the molecule, which causes a remarkable change in their ability to bind to the A-site in the ribosome.[58,59] Aminoglycoside phosphotransferases, also known as kinases, catalyze the regiospecific transfer of the γ-phosphoryl group of ATP to one of the hydroxy groups of the aminoglycoside. The various classes and subclasses of APHs are APH(4)-I, APH(6)-I, APH(9)-I, APH(3′)-I to -VII, APH(2″)-I to -IV, APH(3″)-I, and APH(7″)-I. APH(3′) enzymes are considered to be a major class of the APH family enzymes, which phosphorylate the 3'-hydroxyl of the ring-II in many AGAs. The APH(3') enzyme has been extensively used in molecular biology as traceable resistance marker (*e.g.*, the *neo* cassette).[60] Phosphorylation of the aminoglycosides influences significantly their binding to their target at the A-site of the ribosome. From a clinical point of view the APH(2”) enzyme is the most problematic aminoglycoside phosphotransferase, because it results in high-level resistance to the majority of the clinically used AGAs of the 4,6-class (*e.g.*, gentamicin).[61]

1.5.2.3.3. Aminoglycoside Nucleotidyltransferases (ANT)

Although the ANTs are a relatively small family of AMEs, but they are believed to be the major source of AGA resistance mainly found in Gram-negative clinical pathogens, such as *Enterobacteriaceae* and *Pseudomonas*, which are common organisms in food poisoning and cystic fibrosis.[62] The different classes of ANTs are ANT(6), ANT(9), ANT(4′), ANT(3″), and ANT(2″). The ANTs have significant therapeutic importance because amikacin, gentamycin and tobramycin are influenced by ANT (2"). ANTs catalyze the reaction between Mg-ATP and aminoglycosides to form *O*-adenylylated aminoglycosides.[63,64]

1.5.3. Complexity associated with AGAs chemical syntheses

The outstanding activity of the AGAs against Gram-negative pathogens makes them an attractive starting point to build novel derivatives to deal with MDR pathogens, which are increasingly becoming a health threat. Various synthetic methods allow the specific alteration of AGA scaffolds and the generation of new AG analogues that address the AGA resistance by AMEs and toxicity mechanisms. Although chemical synthesis is a better way to generate a large quantities of AGAs, producing big library of clinically important compounds is often a huge task. Because of the multitude of functional groups and structural complexity, the synthesis of AGAs or the minor alterations of AGA in particular location, requires multiple protecting group manipulations. Thus, these synthetic approaches are quite challenging regardless of how minor the modifications to the compounds.[4]

1.6. Strategies to overcome problems associated with AGAs

The toxicity of aminoglycosides potentially can be minimized by the development of new derivatives that are specific for their target rRNA sequences and can differentiate between bacterial, viral and human targets. Researchers have suggested two possible ways to avoid the resistance due to the presence of aminoglycoside modifying enzymes. One way is to develop inhibitors for the modified enzymes, and the other way is to develop analogues of natural aminoglycosides that evade modification by the modifying enzymes. The development of new analogues of natural aminoglycosides is an appropriate method for the expansion of AGAs and can be accomplished by introduction of new functional groups to hinder the recognition and/or action of resistance enzymes. The most significant long term strategy is to reduce both overuse and misuse of antibacterial agents. The most recently developed AGAs such as amikacin or arbekacin are not affected by modifying enzymes, i.e., they retain their antibacterial activity after

modification.[65] Plazomicin (3) is a next-generation AGA that was obtained through chemical synthesis, appending a hydroxy aminobutyric acid substituent at 1-position of the 2-deoxystreptamine ring and hydroxyethyl chain at 6'-position of sisomicin. Plazomicin belongs to 4,6-disubstistuted AGA family and is blocked by G-1405 methyltransferase. In addition, it confers the ability to evade the all known AMEs except AAC(2')-I. It has completed phase II clinical trials in early 2012, till date neither ototoxicity nor nephrotoxicity was reported in human studies.[66]

1.7. Objective of this project

The goal of this project was the development of efficient AGAs that are less toxic (*i.e.*, more selective) and that circumvent resistance. Apramycin and paromomycin were considered to be ideal substrates from which to develop new derivatives by modifying a certain locations of the molecules. Böttger *et al* have demonstrated that apramycin is the first example of an aminoglycoside antibiotic with reduced ototoxicity yet strong antibiotic activity against a range of clinical infectious diseases including multidrug resistant *Mycobacterium tuberculosis,* it causes only little hair cell damage and hearing loss,[25] as observed in the *ex* vivo murine cochlear explant method and *in* vivo guinea pig auditory brainstem response model.

Further, efforts continue towards finding new active molecules, working with similar methods and ended up with increased selectivity by modification at the 4'- and 4',6'- positions of potent natural pseudotetrasaccharide paromomycin. The idea behind this work is that the toxicity of AGAs is due to the sequence similarity between eukaryotic mitochondrial and bacterial ribosomes, both possess an adenosine at position 1408. Thus, paromomycin makes a hydrogen bond to N(1) of A1408 by ring I of C(6')-OH; this is probably the major interaction of paromomycin with this base. The insight led us to modify ring I, and particularly C(6') position,

leading to variation in the activity of paromomycin. The goal of this project is generate paromomycin analogues by introducing new binding sites at 4' and 6' positions. In particular, this meant constructing a fused ring at 4' and 6' positions, which is analogous to bicyclic ring in apramycin.

1.8. Introduction to apramycin and paromomycin

Apramycin, a typical aminocyclitol antibiotic, is less ototoxic than many AGAs currently in use. It is used to treat many bacterial infections in animals caused by *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae.* It was isolated in 1967 as nebramycin component 2 from the fermentation broths of *Streptomyces tenebrarius*[30]. It is a structurally unique aminoglycoside antibiotic, among all other AGAs, in that it contains the unusual bicyclic aminooctodialdose (8 carbon ring), a mono substituted 2-deoxystreptamine unit as a common core, in addition to a 4-amino-4-deoxy-D-glucose unit (Figure 15).

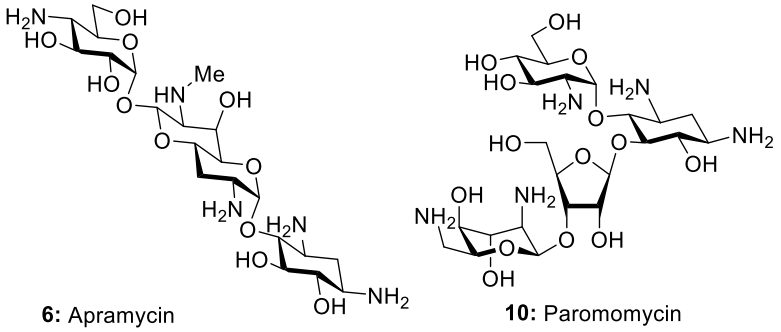


Figure 15: **Chemical structure of apramycin and paromomycin**

Paromomycin is a broad range aminoglycoside antibiotic, active against bacterial strains and protozoa strains, first isolated from *Streptomyces krestomuceticus* in 1950s.[67] It is also known as monomycin or aminosidine and acts as a protein synthesis inhibitor by binding to 16S ribosomal RNA. Paromomycin is a member of the class of 4,5-disubstituted 2-deoxystreptamine aminoglycoside antibiotics (Figure 15). It is out of clinical use as an antibiotic due to its toxicity but was licensed in 2007 in India for the effective and well tolerated treatment of visceral

leishmaniasis (VL) for 21 days (a dose of 11 mg/kg).[67] Paromomycin is listed in list of essential medicines by WHO in 2013.

CHAPTER 2. IMPORTANCE OF THE APRAMYCIN 6'-HYDROXY GROUP AND ITS CONFIGURATION FOR ACTIVITY

2.1. Introduction

As described in the introduction, apramycin is a potent antibiotic with bacterial protein growth inhibitory action against Gram-positive and Gram-negative organisms.[68] This chapter details the synthesis of apramycin derivatives and the influence of these derivatives on antiribosomal and antibacterial activity.

2.2. Synthetic approaches

Apramycin, oxyapramycin, saccharocin and aprosamine derivatives are unique aminocyclitol antibiotics, containing an unusual higher-carbon amino sugar based on the aminooctodiose framework. This higher carbon sugar adopts a dioxa-*trans*-decalin skeleton (Figure 16),[69,70] and, probably as a result of this unique structure, apramycin is less ototoxic than most AGAs used currently and also evades most of the AGA inactivating enzymes.

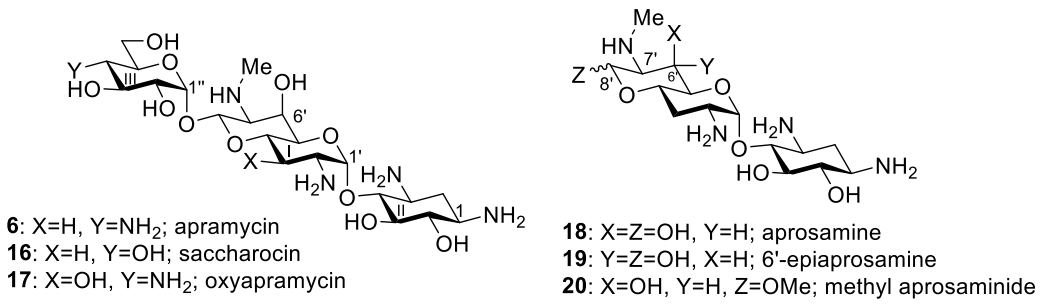
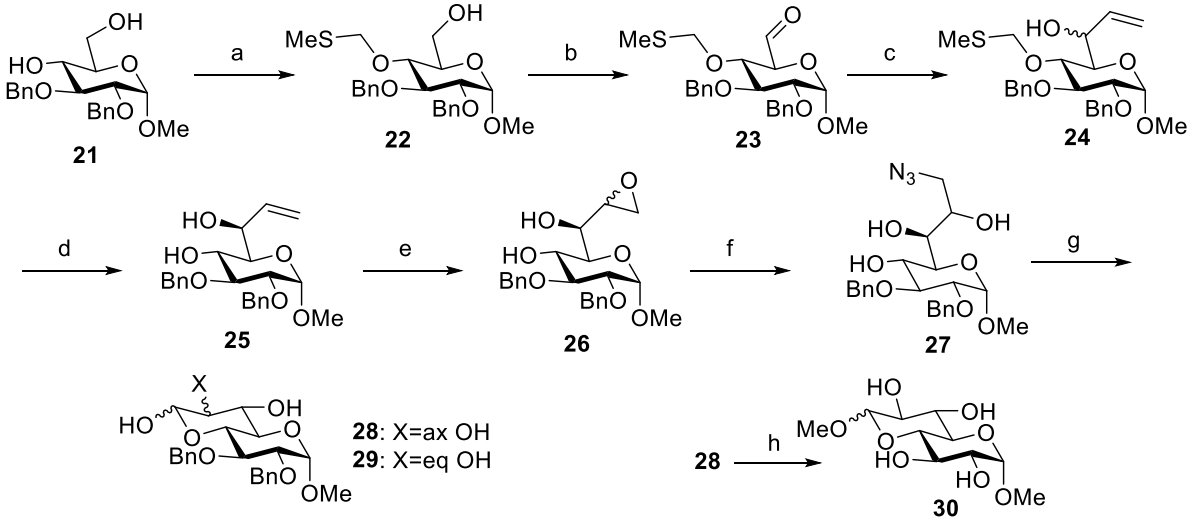


***Figure 16*: Existing apramycin derivatives**

Apramycin and oxyapramycin also are known as nebramycin component 2, and component 7 respectively, and are produced from fermentation broths of *Streptomyces tenebrarius* as first reported in 1967.[71] The skeleton of these AGAs consists of three main features: the rigid bicyclic system (ring I), a 2-deoxystreptamine (ring II) and a 4-amino-4-

deoxy-D-glucose (ring III) as shown in Figure 1. Among the few total syntheses of apramycin, Tatsuta *et al.* reported the first in 1983.[72]

2.2.1. Synthesis of octodiose



a) BzCl, Py, 77%; $MeSCH_2Cl$; NaOMe, 66%: b) DMSO, DCC, TFA, Py, Quant c) Vinylmagnesium bromide, 63%, 5:1: d) MeI, 84%: e) *m*-CPBA, 78%: f) NaN_3,NH_4Cl, 82%: g) hυ, 57%: h) MeOH, Dowex Ion-exchange resin H_2/Pd-C, 84%

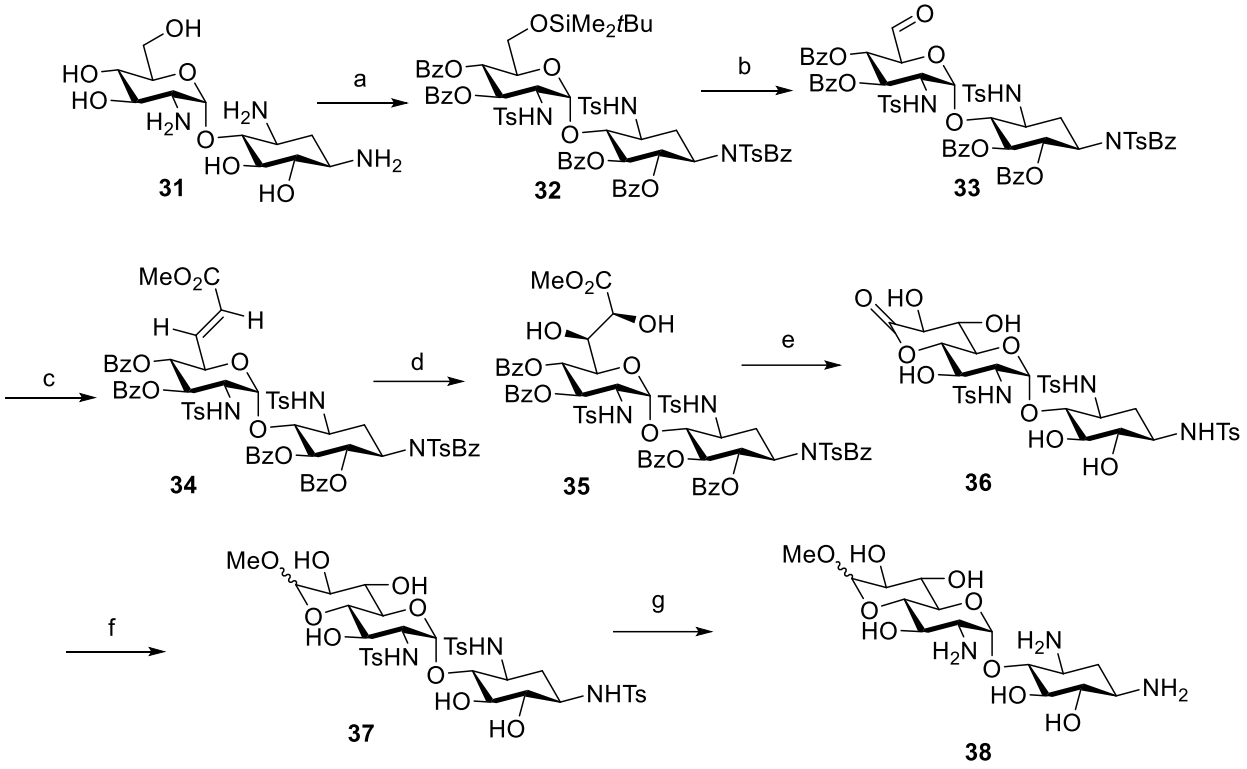
Scheme 1: **Synthesis of octodiose (dioxa-*trans*-decalin structure)**

Leading up to the total syntheses, Szarek and co-workers reported the synthesis of the octodiose dioxa-*trans*-decalin structure from methyl-α-glucopyranoside.[70,73] The synthesis began with selective mono-benzoylation of methyl 2,3-di-*O*-benzyl-α-D-glucopyranoside. Subsequent protection of the secondary hydroxyl group as a methylthiomethyl ether was followed by removal of the benzoyl group at the 6-position and furnished compound **22**. Aldehyde **23**, achieved by oxidation of primary alcohol **22**, was treated with a Grignard reagent affording the epimeric mixture of **24** (in a 5:1 ratio). The major isomer was treated with *m*-CPBA to generate epoxide **26** which, when subjected to a ring opening with sodium azide, gave the epimeric azides **27**. Photolysis of the azide lead to the bicyclic **28**,[74] which on methanolysis yielded a mixture of

glycosides **30**. These scaffolds are considered as the first synthetic examples of dialdoses with the dioxa-*trans*-decalin structure (Scheme 1).[70,73]

2.2.2. Total synthesis of 4-O-(2-amino-2-deoxyoctodiosyl)-2-deoxystreptamine

Later, Szarek *et al.* reported the first total synthesis of 4-*O*-(2-amino-2-deoxyoctodiosyl)-2-deoxystreptamine starting from the disaccharide paromamine **31**, which contains the key glycosidic linkage between 2-deoxystreptamine and the aminoglycosyl moiety. The synthesis began with paromamine **31** which was subjected to *N*-tosylation, then selective silylation of the primary hydroxyl group followed by benzoylation of remaining hydroxyl groups, to give the protected paromamine **32** in a 62% overall yield. The silyl group was removed under mild acidic conditions to give the 6'-alcohol, which was subjected to dimethyl sulfoxide-based oxidation to furnish the key aldehyde intermediate **33** in 85% yield. Treatment of **33** with (ethoxycarbonyl-methylene)triphenylphosphorane afforded the exclusively *E*-α,β-unsaturated octuronic ester **34** in 80% yield. When this ester **34** was subjected to *cis*-hydroxylation using osmium tetroxide it gave ethyl D-*threo*-D-*gluco* octuronate **35** in 80-90% yield (3:1 ratio). Ethyl octuronate **34**, when treated with sodium methoxide, underwent debenzoylation and simultaneous lactonization to an octurono-8',4'-lactone **36**, with a dioxa-*trans*-decalin structure. Partial reduction of uronolactone **36** using lithium aluminium hydride followed by methanolysis gave the corresponding octodiose derivative **37** as a 1:1 mixture (anomers at the 8' position). Finally, cleavage of all tosyl groups was achieved with sodium in ammonia, after which purification by ion-exchange chromatography yielded the free base **38** (Scheme 2).[69]

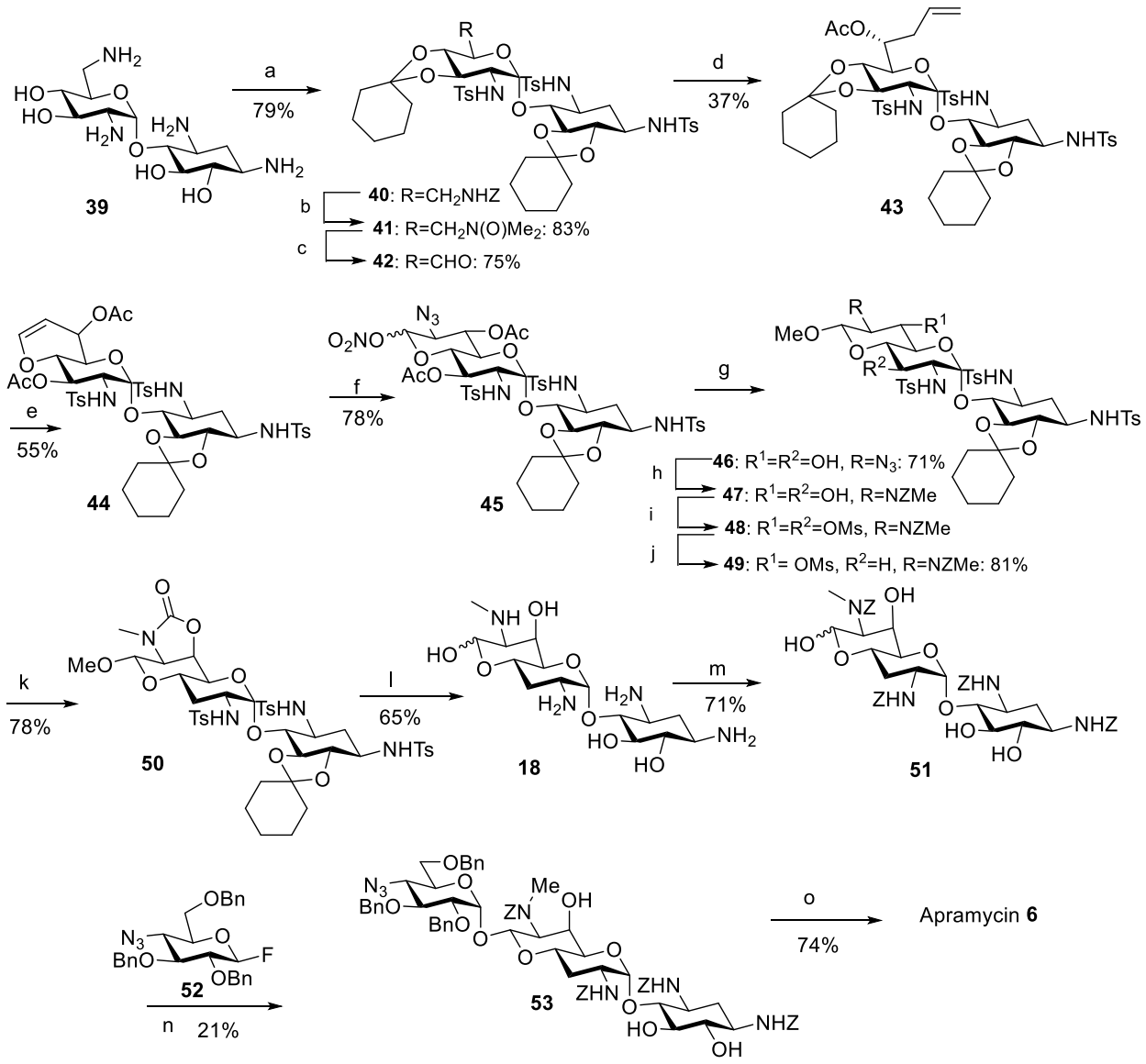


a) TsCl, Na_2CO_3, 86%; $tBuMe_2SiCl$, DMAP, 82%; BzCl, Py, 95%: b) AcOH, 90 °C,80%; DMSO, Py, TFA, 85%: c) (ethoxycarbonyl-methylene)triphenylphosphorane, 80%: d) OsO_4, Py, 66%; e) NaOMe, Quant.: f) $LiAlH_4$, 34%; Methanolic HCl, 86%: g) Na-NH_3, 91%

Scheme 2: **Synthesis of 4-*O*-(2-amino-2-deoxyoctodiosyl)-2-deoxystreptamine**

2.2.3. Total synthesis of apramycin

Tatsuta and co workers reported the first total synthesis of apramycin **6** by a route that also allows the synthesis of a variety of structural analogues. The synthesis commenced with the preparation of the protected compound **40** from neamine **39**, by the following sequence of steps: *N*-benzyloxycarbonylation, *N*-tosylation and *O*-cyclohexylidenation in an overall 79% yield.



a) Z-ONB/Et_3N/aq. MeOH; TsCl/Na_2CO_3/aq. dioxane; 1,1-dimethoxycyclohexane,TsOH: b) KO-t-Bu, t-BuOH; 30% HCHO, $NaBH_3CN$, AcOH: c) m-CPBA; BzCl,i-Pr_2EtN: d) allylmagnesium chloride; Ac_2O/Py: e) OsO_4,$NaIO_4$, aq AcOH; TsCl, TEA, Ac_2O/py: f) NaN_3, CAN: g) $Ba(OH)_2$/MeOH: h) 4 step process see ref: i) MsCl: j) LiCl; Bu_3SnH, AIBN: k) NaOAc: l) Na/liq. NH_3; H^+: m) Z-Cl/Na_2CO_3: n) $SnCl_4$, $AgClO_4$: o) H_2/Pd-block.

Scheme 3: **Synthesis of apramycin**

Saponification of **40** with a base, then reductive amination of 6'-amino derivative followed by oxidation with *m*-CPBA afforded *N*-oxide **41** in 83% overall yield. Compound **41** was treated with benzoyl chloride in the presence of Hünig's base and gave aldehyde **42** in 75% yield. Addition of a Grignard reagent to **42** gave a mixture of alcohols (6'*S* and 6'*R*), and the glycal **44** was achieved in four steps from the 6'*S*-alcohol.

Azidonitration of **43** gave the 7'-azido derivatives in 4:1 ratio (78% yield), then alkaline treatment of the major isomer in methanol afforded methyl β-glycoside **46** in 70% yield. Subsequently, the 7'-*N*-(benzyloxycarbonyl)-methylamino derivative **47** was obtained in a four step process from **46**. The 3'-deoxy compound **49** was achieved by a three step sequence: mesylation, replacement of the labile 3'-mesylate with chloride, and finally radical dehalogenation. Epimerization **49** at the 6'-position group was achieved by the treatment with sodium acetate to give the oxazolidinone **50**. Deprotection of the tosyl groups was accomplished with sodium in liquid ammonia, then alkaline hydrolysis and subsequent acidic hydrolysis provided the aprosamine **18**. Finally, the introduction of the 4-amino-4-deoxy-D-glucosyl scaffold was achieved through the glycosylation of the alcohol **51** with glycosyl donor **52** under modified Mukaiyama conditions. Hydrogenolysis of **53** followed by resin purification furnished apramycin **6** (Scheme 3).[72,75]

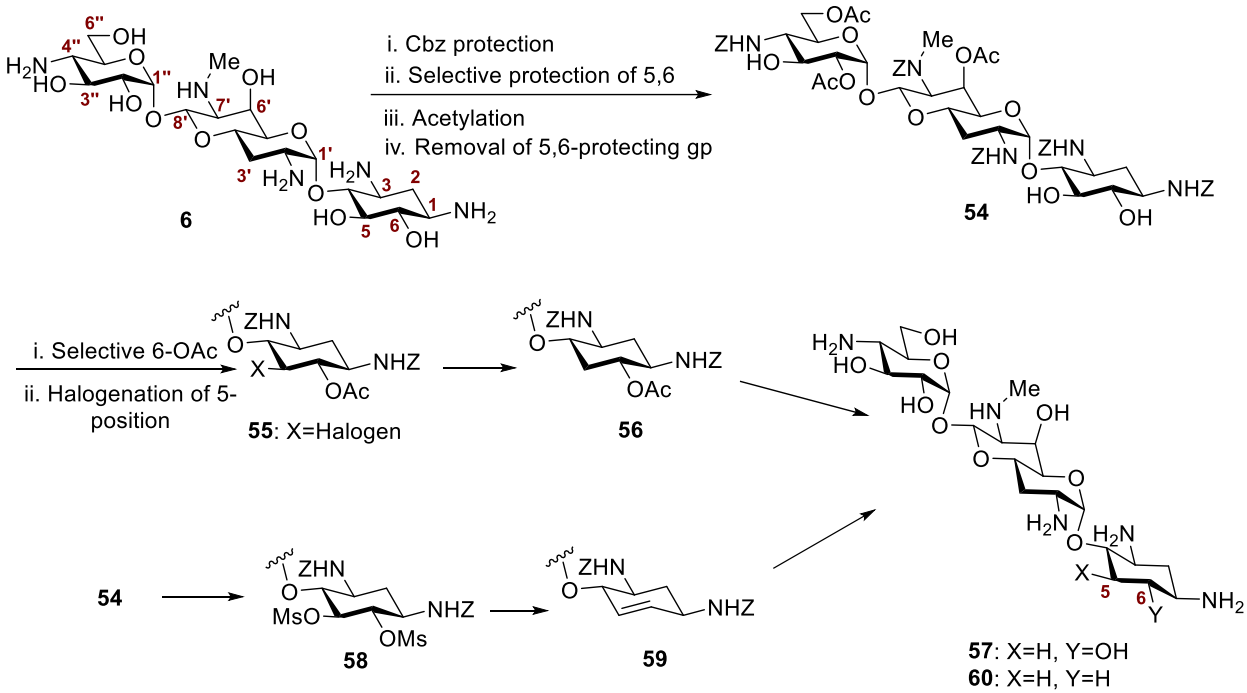
2.3. Existing modifications of apramycin

The existing literature on apramycin covers the influence of modification of functional groups at various locations on its antibacterial activity. Numerous patents have been filed for the development of new apramycin AGAs that can evade resistance and have limited toxicity. Most modifications of apramycin were reported in the 1980s and include 5-deoxy and 5,6-dideoxy derivatives,[76,77] glycosides at the 5- and 6-positions,[78] modification at N1, N2', N7', and N4",[79-82] modification at the O6" position,[83] and the preparation of aprosamine **18** and its methyl β-glycoside **20**.[25,75]

2.3.1. Modification of the 5- and 6- positions of apramycin

A novel method for derivatizing apramycin in one regioselective modification is highlighted in US patents 1982/4,358,585 and 1983/4,370,475. The synthesis of 5-deoxy and

5,6-dideoxyapramycin is achieved by installation and then reductive removal of halogen functionality in apramycin. The resulting apramycin analogue had reinforced antimicrobial activity against Gram positive and negative bacteria pathogens.

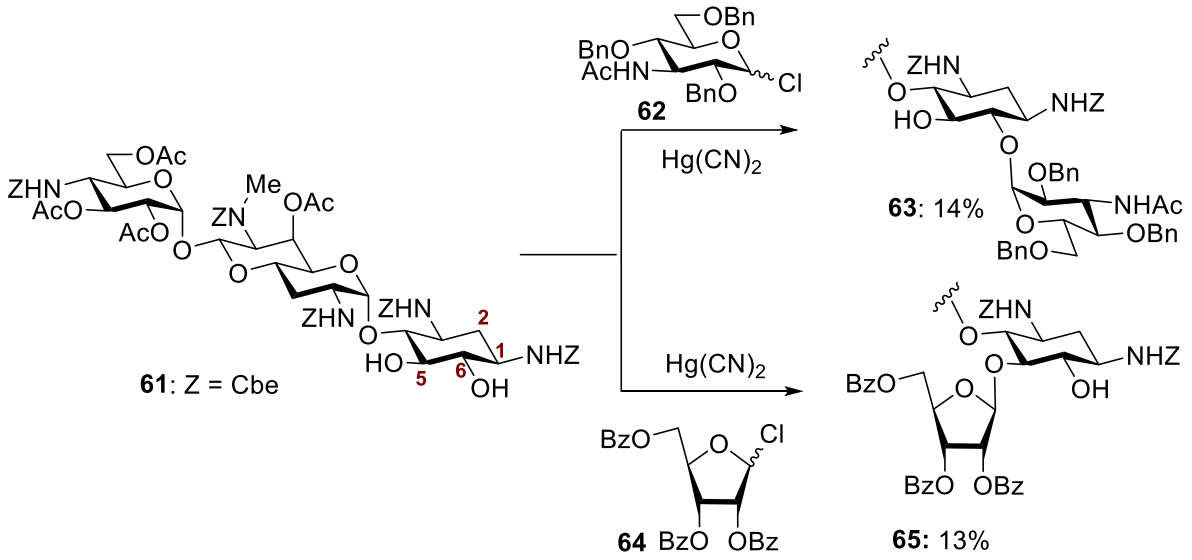


Scheme.4: **Approaches to the synthesis of 5-deoxy and 5,6-dideoxyapramycin**

The modification of 5- and 6-positions of apramycin involved a four step sequence to achieve 5,6-diol intermediate **54** beginning with the carbamate protection of all amines followed by masking of the 5,6-hydroxyl groups with a cyclic acetal. Then, the remaining hydroxyl groups were protected as esters followed by acidic hydrolysis of the acetal to give the key 5,6-diol **54**. Selective 6-*O*-acetylation was followed by introduction of halogen at the 5'-position; reductive removal of the halogen gave **56**. Global deprotection by hydrolysis and catalytic hydrogenation afforded 5-deoxyapramycin **57**. Further, the key intermediate **54** was subjected to sulfonylation with methanesulfonyl chloride followed by reaction of sodium iodide and zinc dust to provide

the 5,6-dideoxy intermediate **59** with a 5,6-double bond. Hydrolysis and catalytic hydrogenation then gave the 5,6-dideoxyapramycin derivative **60** (Scheme 4).[76,77]

In an attempt to maximize activity by incorporating ring II of the 4,5- or 4,6-AGAs, Kawaguchi *et al.* have also reported the synthesis of 5-*O*-β-D-ribofuranosyl apramycin **65** and 6-*O*-(3-amino-3-deoxy-α-D-glucosyl) apramycin derivative **63** (Scheme 5) and these compounds were tested for activity against bacterial organisms and strains producing AMEs.[78]

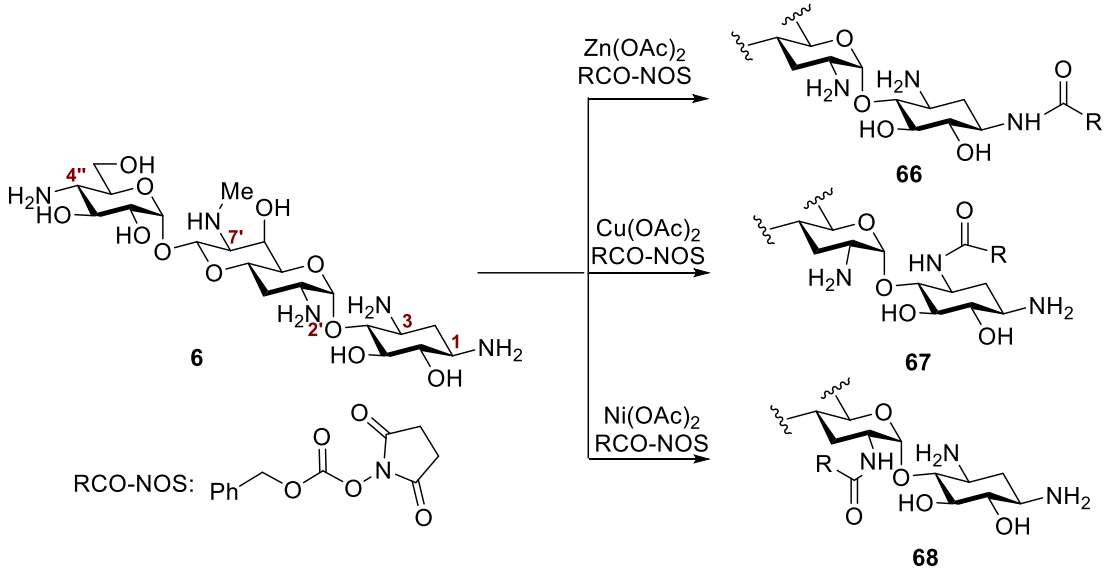


Scheme 5: **Synthesis of 5-*O*-β-D-ribofuranosyl apramycin and 6-*O*-(3-amino-3-deoxy-α-D-glucosyl) apramycin derivatives**

2.3.2. Modification of the *N*1, *N*3, *N*2', *N*7', and *N*4''- positions of apramycin

Novel methods for derivatizing apramycin by regioselective modification are disclosed in various US patents dating from 1982-84. Kirst and co-workers reported that the synthesis of *N*1, *N*3, and *N*2'-derivatives of apramycin can be simply achieved by transition metal-directed acylations of apramycin and related AGAs.[79,80,84,85] They selectively protected amino functionality by changing the transition metal cations. Zinc salts are used to achieve regio-selective acylation and alkylation of N1 of 4-*O*-substituted-2-deoxystreptamine AGAs in a single

reaction. Copper salts are used for the synthesis of N3-acyl derivatives of apramycin in a single step. Nickel salts are used to accomplished the regioselective acylation and alkylation of N2' of 4-*O*-substituted-2-deoxystreptamine containing AGAs (Scheme 6). These methods were used to incorporate numerous alkyl chains including C2-C4 alkyl derivatives. Acyl derivatives were subjected to diborane or lithium aluminium hydride reduction to access the corresponding alkyl chains. The resulting derivatives were tested for antimicrobial activity against Gram positive and negative bacteria pathogens.



Scheme 6: **Transition metal directed derivatization**

A new approach for regioselective derivatization of apramycin, in particular at *N*4" position was highlighted in the US patent 4,360,665.[81] The protection strategy for selective modification at 4" position involves masking of all amine functional groups with a benzyloxycarbonyl protecting group, after which the 5,6- and 2",3"- diols were each protected as isopropylidine acetals. Finally, the 6"-hydroxyl group was protected as in the form of C1-C4 esters. These derivatizations set the stage for the key step of this method, which involves the hydrogenation or base hydrolysis to provide the 4"-free amine so that migration of the 6"-*O*-acyl

group can give the 4''-derivative of apramycin. 4''-*N*-alkyl analogues were then prepared by reduction of corresponding acyl derivatives using diborane or lithium aluminum hydride.[81]

The synthesis of 7'-*N*-alkylapramycin derivatives and their biological activity is reported in the US patent 4,458,065.[82] 1,3,2',4''-Tetra-*N*-protected apramycin derivatives were accessed by first making an apramycin-carbon dioxide complex. Then, the 7'-*N*-alkyl-1,3,2',4''-tetra-*N*-protected apramycin derivatives were prepared by alkylation. General deprotection strategies yielded the 7'-*N*-alkyl derivatives, whose antibacterial activity is similar to that of the parent apramycin.

2.3.3. Modification of the 6''-position of apramycin

In US patent 4,379,917,[83] the synthesis of apramycin derivatives altered at the 6''-position with a range of substituents was reported together with their antibacterial activity. The synthesis of 6''-substituted-apramycin antibiotics can achieved by protecting the five amines with carbamate protecting groups, and the 5,6- and 2'',3''-diols with cyclohexylidene or isopropylidene acetals. The 6''-hydroxyl group is then substituted with by variety of substituents including halogen, thio, azido, cyano, etc. Subsequent deprotection, accomplished by either basic hydrolysis, acidic hydrolysis or hydrogenation over a palladium catalyst, gave the targeted 6''-derivatives.

2.4. Choice of apramycin as parent

Cell free translation assays with single point and hybrid ribosomes described by the Böttger group predict low toxicity for apramycin, which is borne out in cochlea explant studies showing little hair cell loss and eventually in guinea pig models. Furthermore, MIC studies with bacteria carrying the various AMEs show apramycin to not be affected by them. Thus, apramycin is potentially a excellent AGA except that it generally has weaker activity than AGAs

currently in clinical use. Apramycin is relatively unsusceptible to modification by AMEs compared with competitor AGAs. Specifically, only the AACs modifying positions *N*1 and *N*3 by acylation are effective. Furthermore, in contrast to most AGAs apramycin is active against *Enterobacteriacae* carrying genes for the 16S rRNA methyltransferases.[86] Overall, the findings of the lack of ototoxicity and minimal resistance in bacteria carrying AMEs and methyltransferases, make apramycin a good lead for further improvement.

2.5. Rationale

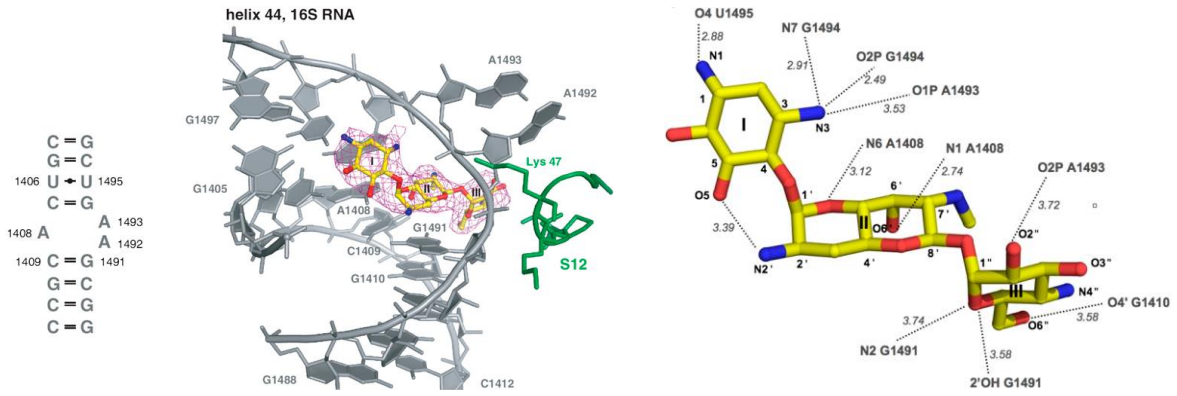


Figure 17: **The interactions between apramycin and 16S rRNA nucleotides.** (This figure has been reproduced from "Matt, T.; Ng, C. L.; Lang, K.; Sha, S.-H.; Akbergenov, R.; Shcherbakov, D.; Meyer, M.; Duscha, S.; Xie, J.; Dubbaka, S. R.; Perez-Fernandez, D.; Vasella, A.; Ramakrishnan, V.; Schacht, J.; Böttger, E. C., *Proc Natl Acad Sci.* **2012**, *109*, 10984-10989".)

As described in chapter 1, Section 1.4.2, in X-ray studies with the complete 30S bacterial ribosomal subunit the bicyclic ring II of apramycin is bound to the flipped out conformation of the bacterial ribosomal A site similar to ring I of the 4,5- and 4,6-AGAs. The β-face of ring II interacts through CH-π interactions with the bacterial ribosomal base G1491, and sugar-base-pair interactions are formed with the universally conserved A1408 of the bacterial ribosomal A site. In particular, the 6'-OH group of AGA serves as hydrogen bond donor to *N*1 of the ribosomal base A1408 and the ring oxygen (O5') acts as hydrogen bond acceptor from the *N*6 amine of A1408 (Figure 17).[26] However, crystallographic studies of apramycin bound to a short model

sequence corresponding to the eukaryotic ribosomal A site have the drug bound to a flipped in conformation in which A1492 forms hydrogen bonds with G1408. In this complex, the bicyclic ring II of apramycin is rotated with respect to its orientation in the bacterial complex and does not stack with A1491. The 2'-NH_2 group of apramycin forms a hydrogen bond to the G1408 O6, which is free from any interactions in the bacterial complex (Figure 17). Moreover, apramycin O5' and O6' bind to three of the six hydration water molecules of a magnesium ion, rather than to the base at position 1408 i.e., the apramycin 6'-OH is exposed to water.[29,30] There is therefore a dichotomy between the modes of binding of apramycin to the complete bacterial 30S subunit and the short eukaryotic model sequence, leading to uncertainty about the true binding mode. Modification of the 6'- and 7'- positions of apramycin was considered to be a suitable approach to address this dichotomy and shed light on the correct binding mode. The following sections discus work conducted to modify the 6' and 7' positions and their effect on antiribosomal and antibacterial activity.

2.6. Results and discussion

Various modifications can be made at the 6'-position including inversion of the hydroxyl group, replacement of hydroxyl group with both inversion and retention by an amine group, replacement by a halogen atom and by a hydrogen atom. Modifications can also be made at the 7'-position including the preparation of analogues in which the methyl group is replaced by longer alkyl chains. Other possible modifications at the 7'-position include desamino, desmethyl and hydroxyl analogues (Figure 18).

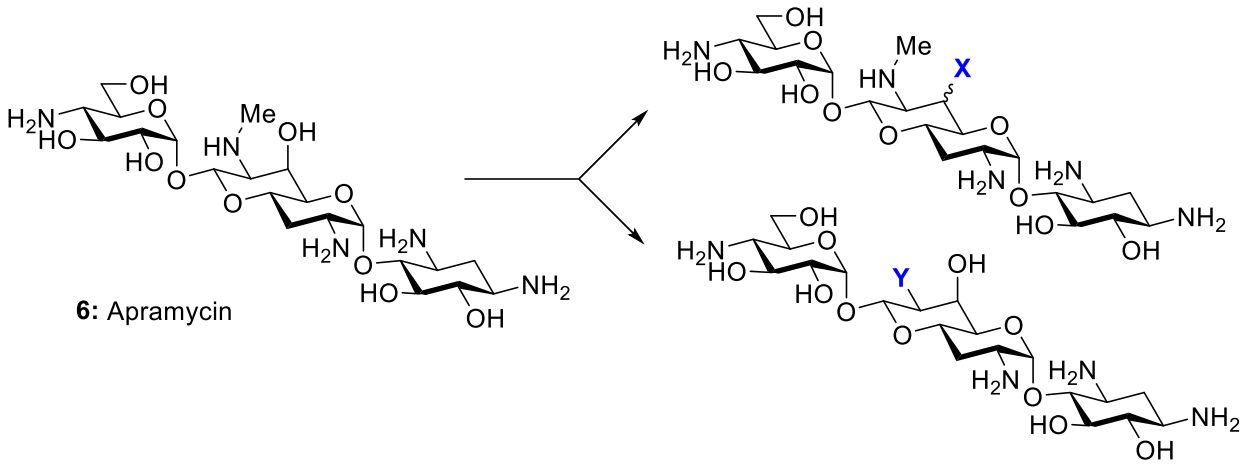


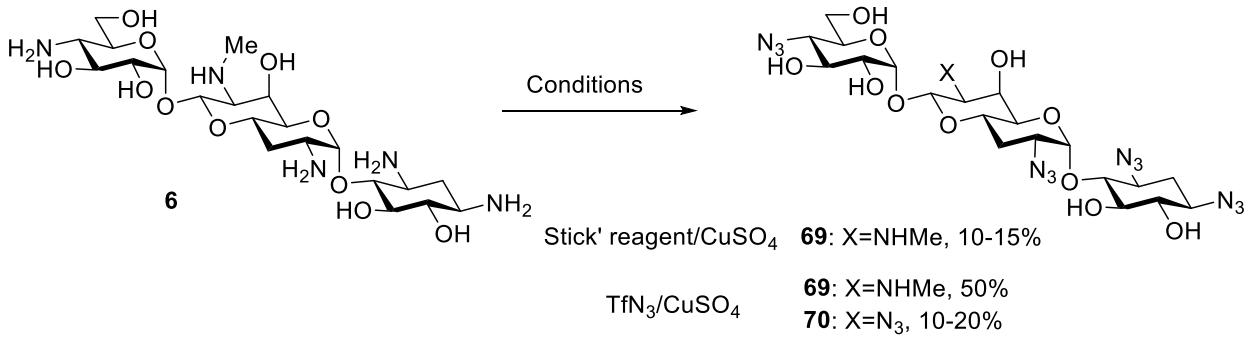
Figure 18: **Apramycin analogs targeted**

2.6.1. Modification at the 6'-position

After several unsatisfactory approaches using carbamate-based strategies, all primary amines were protected as azides and the secondary amine as a benzyl carbamate. Initially, the 5,6- and 2",3"-*trans*-vicinal diols were protected as Ley-type bisacetals, but this was found difficult to cleave. Eventually, as described below, selective protection of the 6'-alcohol was achieved by oxazolidinone formation.

2.6.1.1. Synthesis of a key apramycin protected intermediate

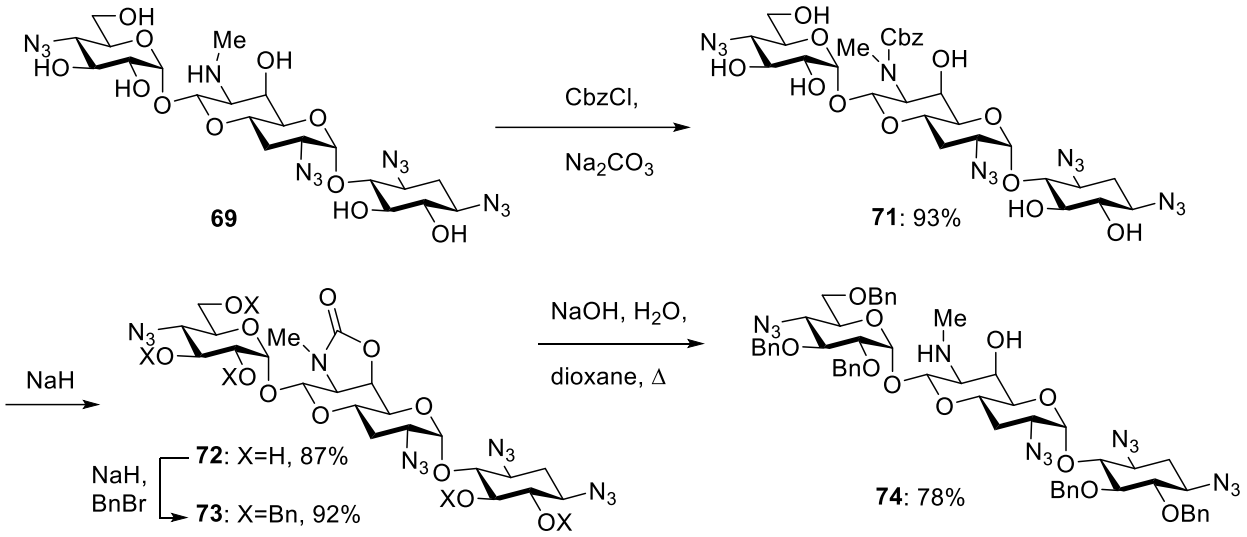
The synthesis commenced with primary amine conversion to azides by copper-catalyzed diazotransfer reaction using imidazole-1-sulfononyl azide hydrochloride, also known as Stick's reagent,[87] a commonly used diazotransfer reagent. Unfortunately, this reagent afforded the desired 1,3,2',4"-tetraazido derivative **69** in only 10-15% yield.



Scheme 7: **Synthesis of azide protected apramycin derivatives**

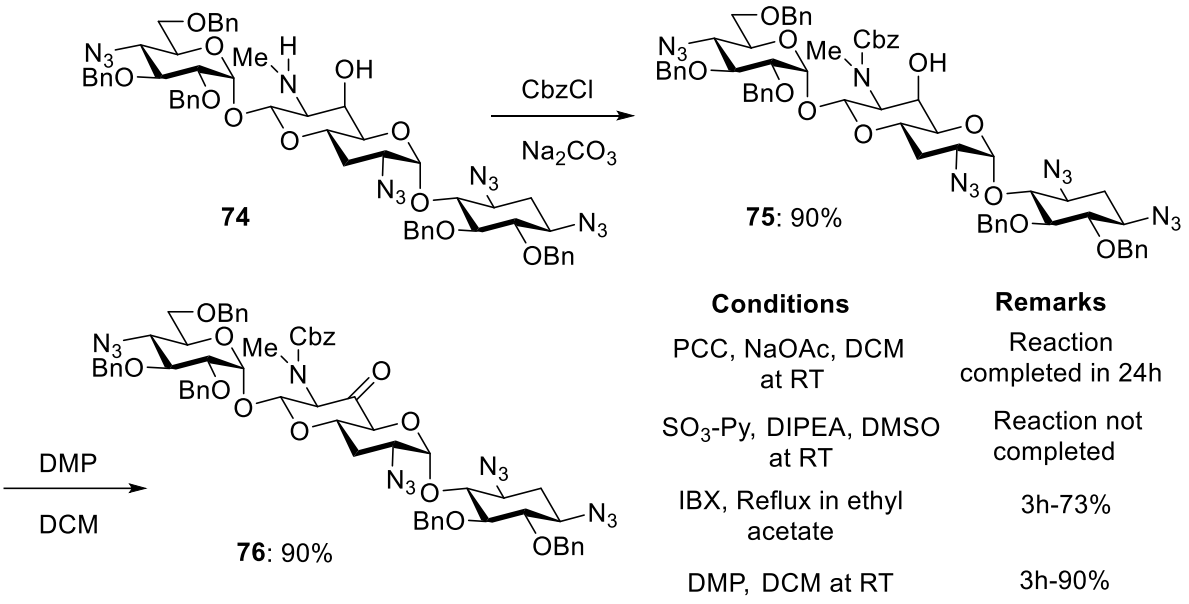
On the other hand, reaction of apramycin sulfate **6** with trifluoromethanesulfonyl azide gave 1,3,2',4''-tetraazido derivative **69** together with the 7'-demethyl-1,3,2',7',4''-penta-azido compound **70** in 50% and 10-20% yield, respectively (Scheme 7).[88] Trifluoromethanesulfonyl azide, which can be synthesized in situ by reaction of sodium azide and trifluoromethanesulfonic anhydride,[89] was therefore the reagent of choice. Demethylated compound **70** was separated and the influence of its *N*7'-methyl group on activity of apramycin was analyzed.

2.6.1.2. Synthesis of 6'-epi, 6'-deoxy and 6'-methyl apramycin intermediates



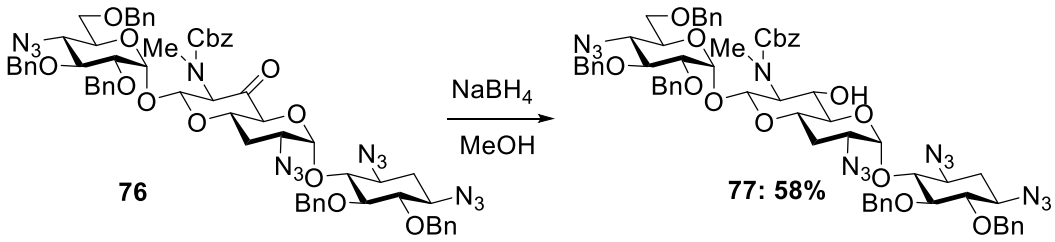
Scheme 8: **Synthesis of protected apramycin intermediate (except 6', 7')**

Subsequently, carbamate formation at the secondary amine with CbzCl gave **71** in 93% yield with a 4:3 ratio of rotamers as determined by ^{1}H NMR. Treatment with sodium hydride then provided the 6',7'-oxazolidinone **72** in 87% yield. Benzylation of all the remaining hydroxyl groups using benzyl bromide in the presence of sodium hydride gave **73** in 92% yield, and was followed by the cleavage of the oxazolidinone ring. This was achieved by heating to reflux with sodium hydroxide in aqueous dioxane and led to the common intermediate **74** in 78% that allowed the subsequent selective facile modifications at the 6'- and 7'-positions (Scheme 8).



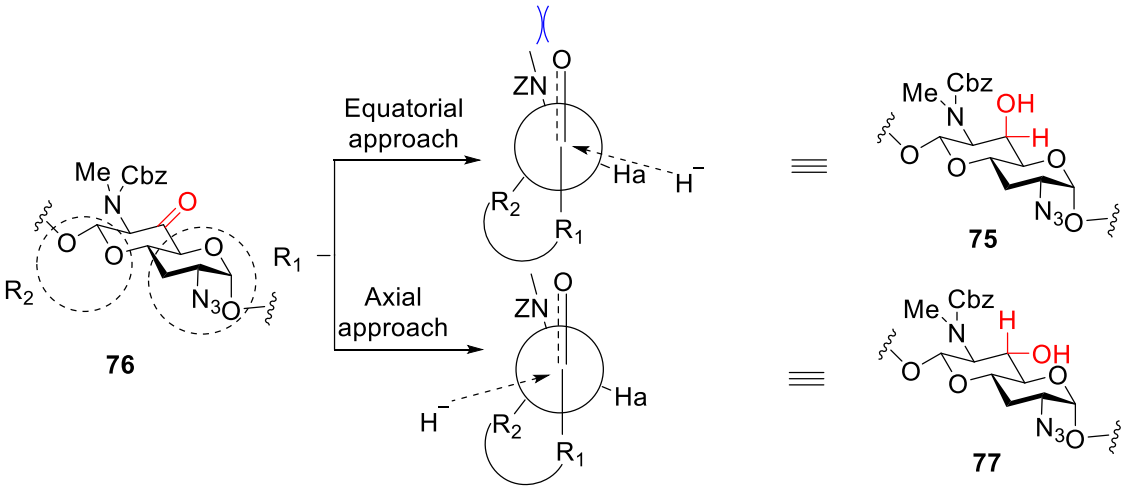
Scheme 9: **Synthesis of 6'-ketoapramycin intermediate and attempts for oxidation shown**

Thereafter, reintroduction of the carbamate group on the secondary amine gave **75** in 90%, with a 3:2 ratio of rotamers according to the ^{1}H NMR data. Afterwards, PCC and SO_3-Py oxidations did not give encouraging results, prompting a switch to hypervalent iodine oxidation to convert the 6'-hydroxy to 6'-keto derivative. IBX was used as an oxidizing reagent but due to the harsh conditions (reflux in ethyl acetate), it was replaced with Dess Martin periodinane[90] for oxidation of 6'-hydroxy group, which afforded the 6'-ketone **76** in 90% yield (Scheme 9).



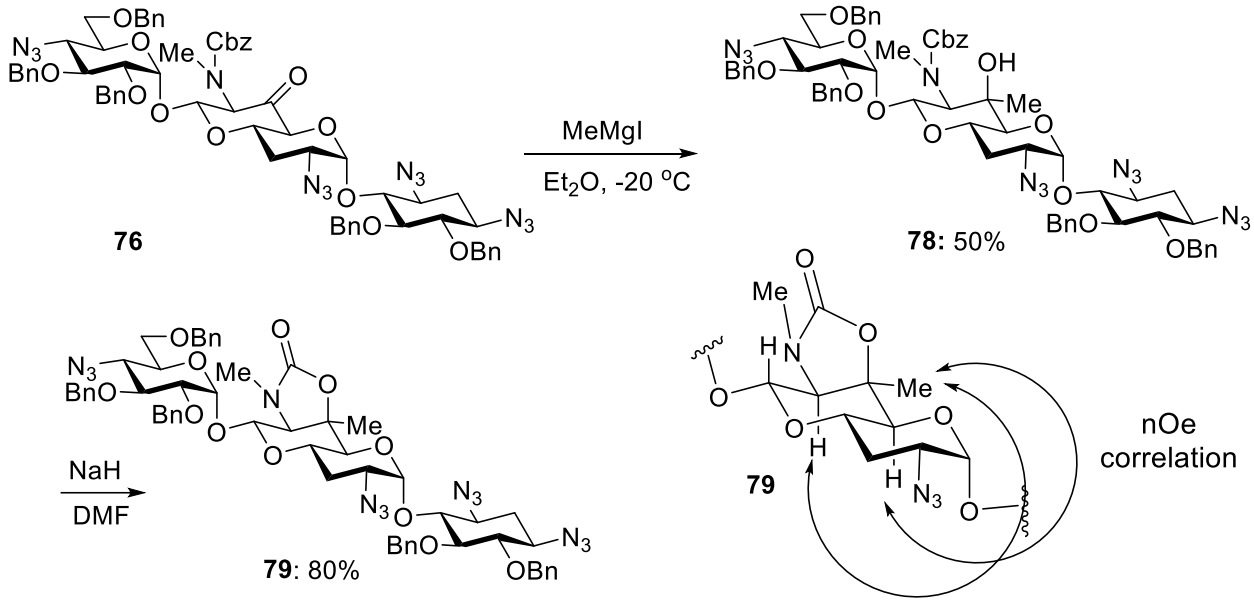
Scheme 10: **Synthesis of 6'-*epi*-apramycin intermediate**

Ketone **76** was subjected to reduction with sodium borohydride in methanol when it gave the 6'-*epi*-apramycin derivative **77** as a separable 5:1 mixture with **75** in 58% yield (Scheme 10). The excellent equatorial selectivity observed in the reduction of the ketone **75** can be explained using the Felkin model as shown Scheme 11. With the relatively compact nucleophile borohydride the 1,3-diaxial interactions to approach along the axial direction are minimal, whereas equatorial approach is disfavored by the developing torsional strain between the incipient alcohol and the adjacent substituent: axial attack is therefore preferred and the equatorial product predominates (Scheme 11).[91,92]



Scheme 11: **Felkin model for hydride reduction of ketone 75**

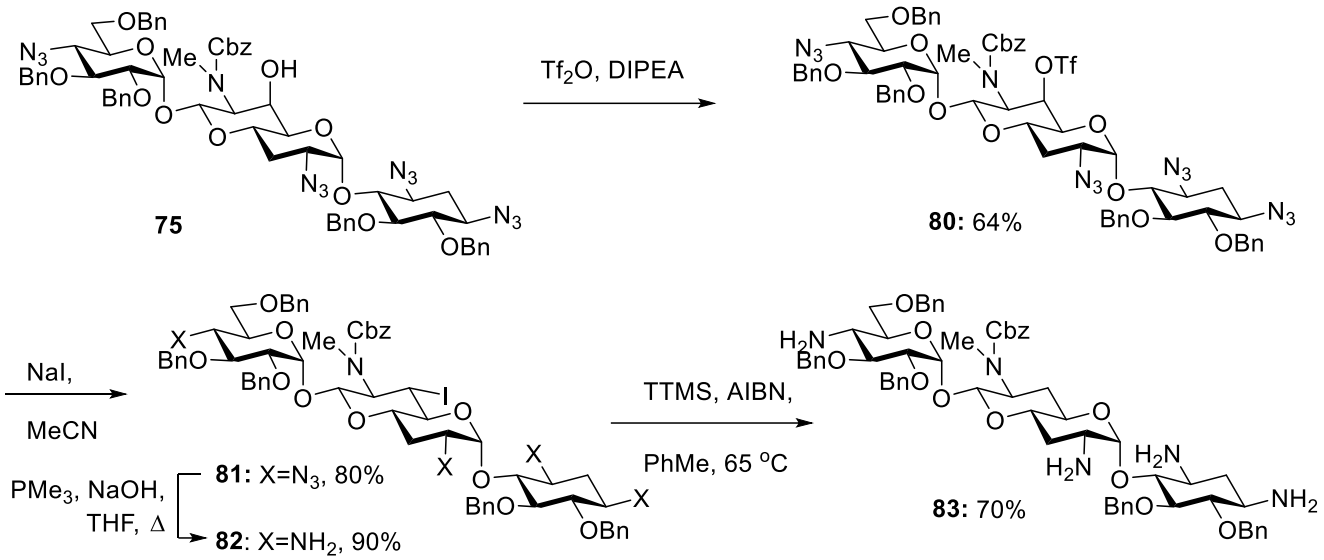
The addition reaction of freshly prepared methyl magnesium iodide to ketone **76** at -20 °C proceeded well to deliver a 6'-methyl apramycin derivative **78** as a single isomer in 50% yield. Due to the rotamer problem, the configuration of **78** was assigned by conversion to the oxazolidinone **79** by treatment with NaH in DMF. The nOe spectrum of **79** showed clear enhancement of the resonances for H-5' and H-7' but not of H-8' on irradiation of the 6'-Me group (Scheme 12).



Scheme 12: **Synthesis of 6'-methyl derivative of apramycin**

The equatorial selectivity of nucleophile addition is explained by the bulk of the nucleophile and the 1,3-diaxial interactions it encounters on axial attack, resulting in preferential equatorial attack.

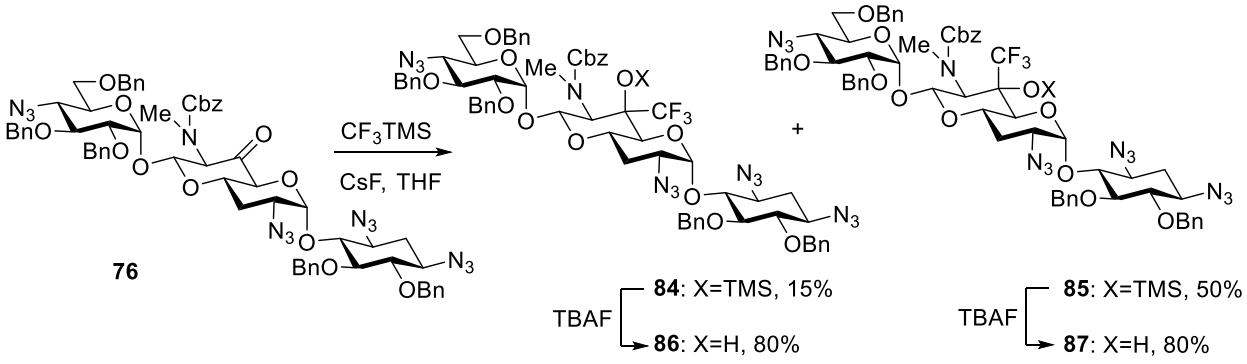
Triflation of **75** with triflic anhydride in DCM gave **80** in 64% yield, and was followed by displacement with sodium iodide which afforded the 6'-deoxy-6'-epi-iodo apramycin derivative **81**. Attempted conversion of the 6'-deoxy-6'-*epi*-iodo compound to the 6'-deoxy compound using AIBN and tris(trimethylsilyl)silane[93] failed because of competing reduction of one or more of the azides. Accordingly, the azides were first reduced with trimethylphosphine leading to the amino compound **82**, which was subsequently treated with tris(trimethylsilyl)silane[93] and AIBN resulting overall in conversion of the iodo compound into the desired 6'-deoxyapramycin intermediate **83** (Scheme 13).



Scheme 13: **Synthesis of a 6'-Deoxyapramycin intermediate**

2.6.1.3. Synthesis of 6'-trifluoromethyl apramycin derivatives

Installation of the trifluoromethyl group was achieved by reaction of **76** with the Ruppert-Prakash reagent[94,95] in the presence of cesium fluoride and gave a 1:3 mixture of the adducts **84** and **85** in 65% yield. The configuration of the trifluoromethyl compounds **84** and **85** was assigned using a $^{13}C,^{1}H$ scalar coupling method[96] that was developed for the purpose, and was confirmed following complete deprotection. On exposure to tetrabutylammonium fluoride, **84** and **85** both gave 80% of the corresponding trifluoromethyl bearing tertiary alcohols **86** and **87** (Scheme 14).

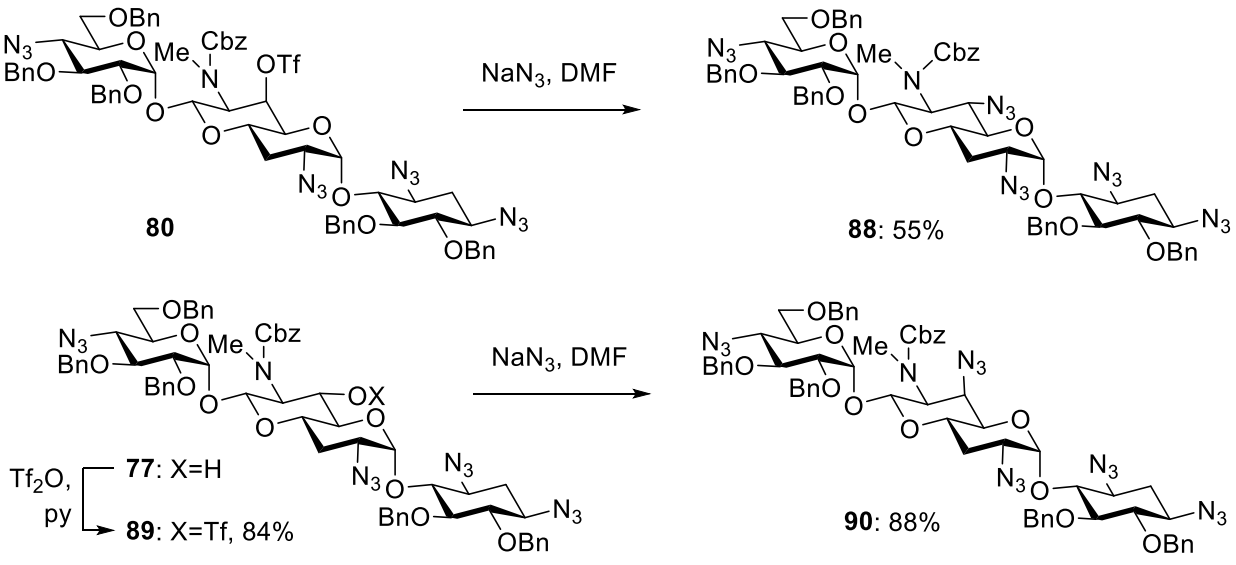


Scheme 14: **Synthesis of 6'-trifluoromethylapramycin derivatives**

The introduction of the CF_3 group to the ketone **76** proceeds with modest axial selectivity which can be explained by the Felkin model similar to that shown for the reduction of the ketone (Scheme 11). Thus, when the CF_3 moiety attacks on equatorial side, significant torsional strain is generated between the newly formed trimethylsiloxyl functionality and the vicinal substituent.[97] As a result, axial attack is preferred although CF_3 is a bulkier nucleophile than the methyl Grignard reagent. This modest axial selectivity can also explained based on the formation of a penta co-ordinate species $R_2CO\text{-}SiMe_3\text{-}CF_3^-$ in which the bulky silyl complex is oriented in equatorial site, as a result axial attack has preference.[97]

2.6.1.4. Synthesis of 6'-deoxy-6'-azido and 6'-epi-6'-deoxy-6'-azido apramycin derivatives

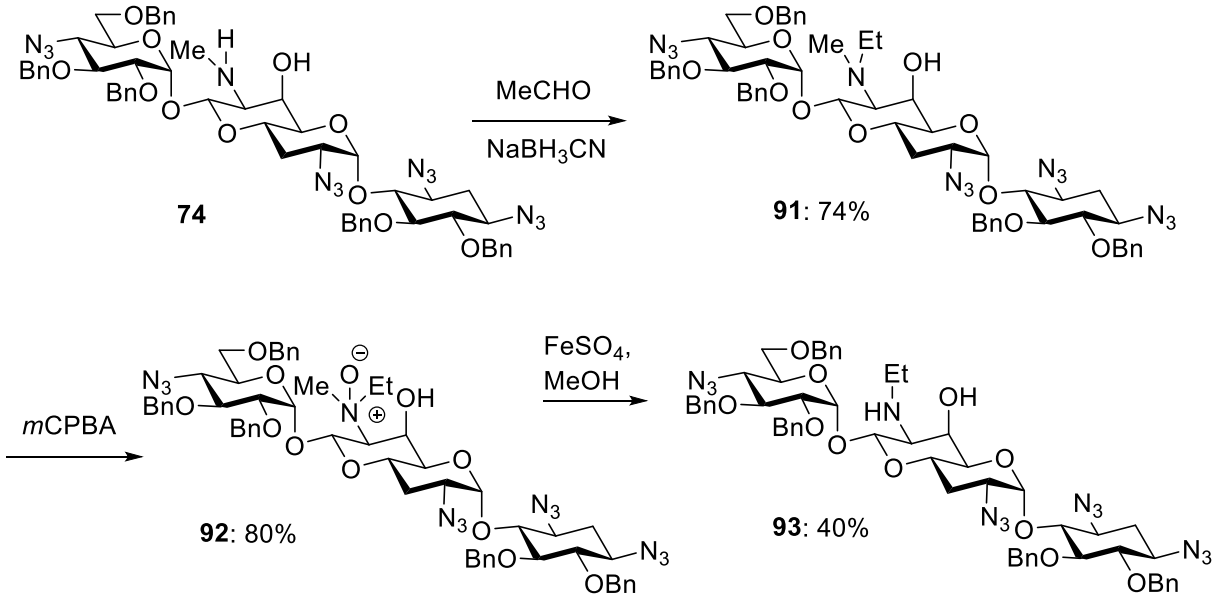
The 6'-epi-6'-deoxy-6'-azido apramycin derivative **88** was obtained from the 6'-triflate **80** by displacement with sodium azide in DMF. The 6'-deoxy-6'-azido apramycin derivative **90** was accessed from the corresponding inverted 6'-triflate **89**, by displacement with sodium azide in DMF (Scheme 15).



Scheme 15: **Synthesis of 6'-deoxy-6'-azido and 6'-epi-6'-deoxy-6'-azido apramycin derivatives**

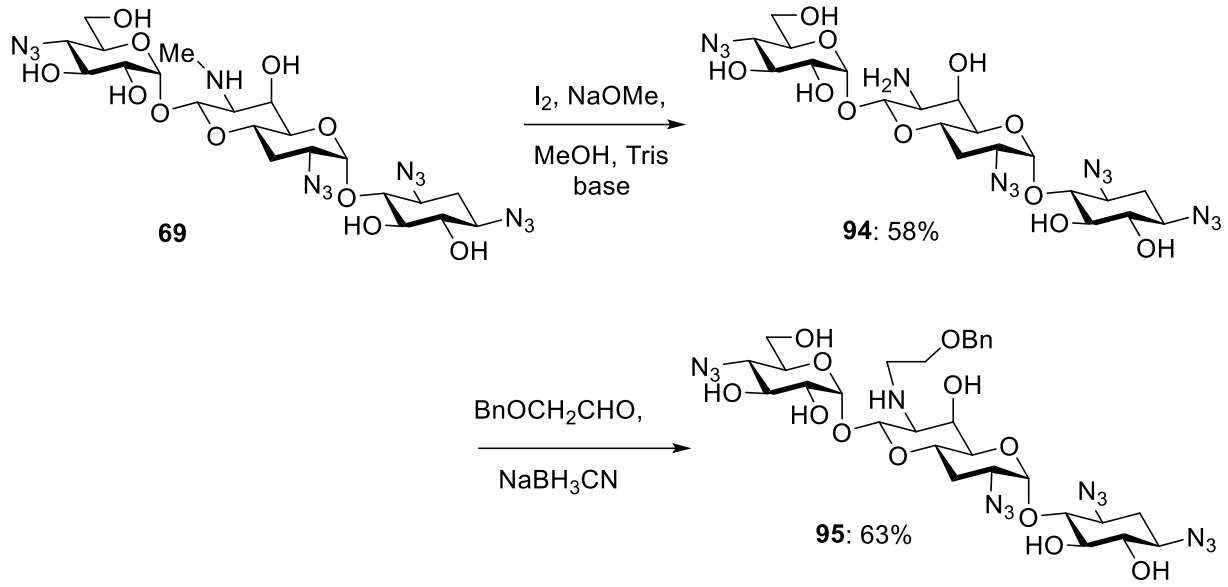
2.6.2. Modification at the 7'*N*-position

In order to modify the 7'-position the protected apramycin intermediate **74** was used as a starting material. Reductive amination of the secondary amine **74** with acetaldehyde and sodium cyanoborohydride gave the **7**'-*N*-ethyl derivative of apramycin **91** in 74% yield.[98] Subsequently *N*-oxide **92** was accessed by treatment with *m*-CPBA in 80% yield as an approximately 1:1 mixture of diastereomers. The *N*-oxide **92** was subjected to demethylation with ferrous sulfate (non-classical Polonovski approach) in methanol, which afforded the 7'*N*-demethyl-7'*N*-ethyl apramycin derivative **93** in 40% yield (Scheme 16).[99]



Scheme 16: **Preparation of 7'*N*-ethylapramycin intermediate**

An alternative demethylation reaction was used to modify at the 7'-position of the secondary amine **69**. In this reaction **69** was subjected to iodine and sodium methoxide in the presence of Tris-base[100] resulting in the formation of the 7'*N*-desmethyl derivative **94** in 58% yield. Reductive amination with benzyloxyacetaldehyde and sodium cyanoborohydride afforded the 7'-*N*-desmethyl-7'-*N*-benzyloxyethyl derivative **95** in 63% yield (Scheme 17).

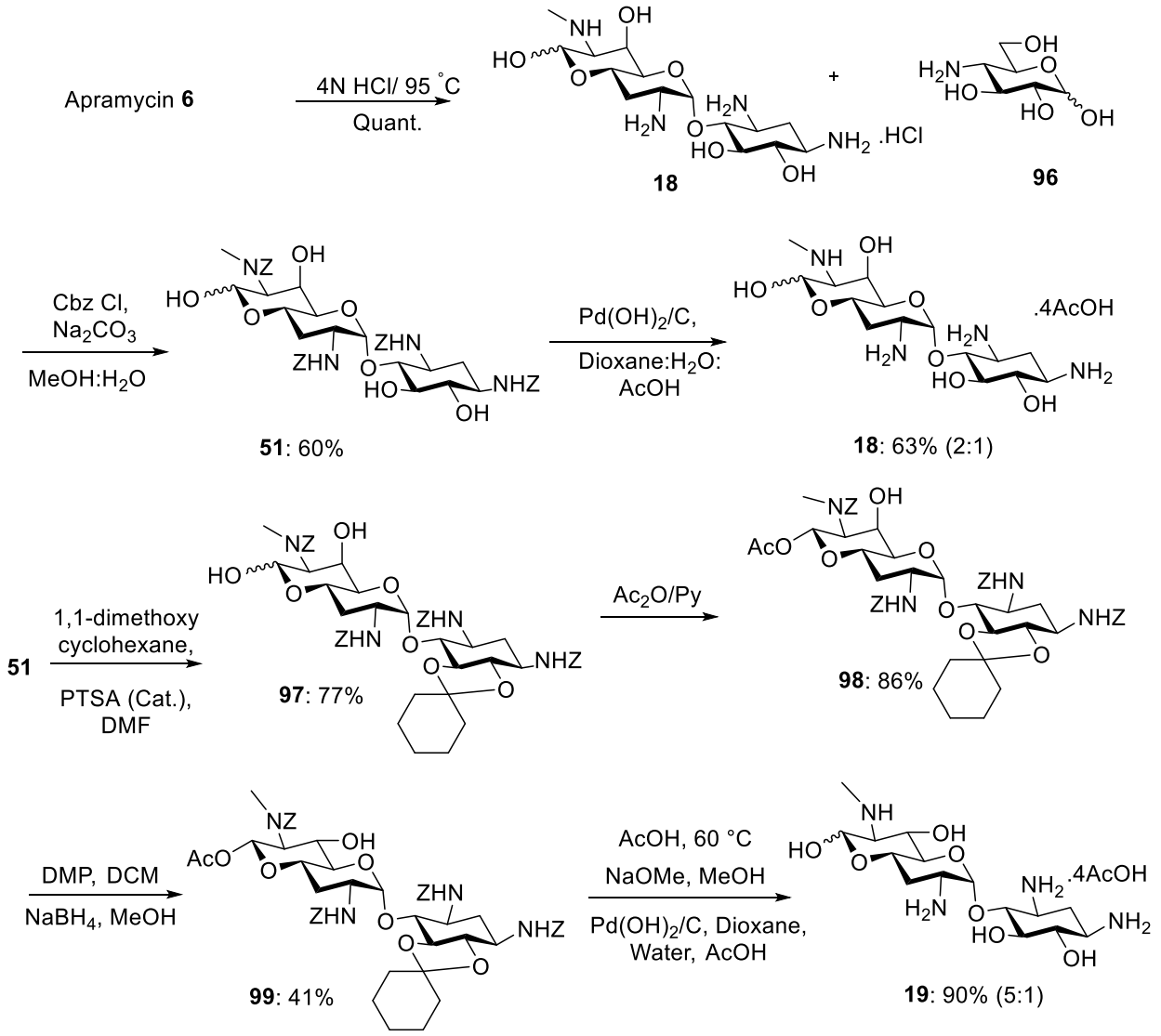


Scheme 17: **Preparation of 7'*N*-desmethyl-7'*N*-benzyloxyethyl apramycin intermediate**

Of the two different approaches used to achieve the *N*-demethylation of apramycin (Schemes 16 and 17) the second is preferred as it directly gave the demethylated derivative with moderate yield. Moreover, this method has broader scope for derivatization at the 7'-position of apramycin.

2.6.3. Synthesis of aprosamine and 6'-epi-aprosamine

Adapting the literature method[71,101] removal of the 4-amino-4-deoxy-D-glucopyranose (ring III) leads to the aprosamine derivatives. The synthesis of aprosamine commenced with acidic hydrolysis of apramycin at 95 ºC providing the aprosamine **18** and 4-amino-4-deoxy-D-glucopyranose hydrochloride as an inseparable mixture. Subsequently, to separate these compounds, all the amines were converted to carbamates. Then, the purification of this mixture provided the carbamate protected aprosamine intermediate **51** in 60% yield. Thereafter, carbamates were removed by conventional hydrogenolysis over palladium hydroxide on carbon at atmospheric pressure in aqueous dioxane in the presence of acetic acid giving aprosamine **18** as the acetate salt in 63% yield (2:1 ratio of anomers at the 8'-position) (Scheme 18).



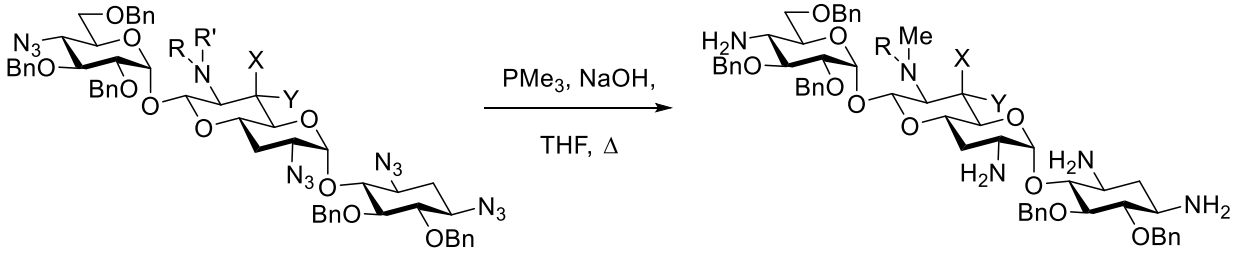
***Scheme 18*: Synthesis of aprosamine and 6'-epiaprosamine**

In the second arm of the scheme, reaction of the aprosamine intermediate **51** with 1,1-dimethoxycyclohexane in the presence of a catalytic amount of an acid gave the 5,6- protected aprosamine intermediate **97** in 77% yield. Selective acetylation of **97** with acetic anhydride in pyridine gave **98** in 86% yield, which was subjected to oxidation with the Dess Martin periodinane followed by the reduction with $NaBH_4$ to give the 6'-epi-aprosamine derivative **99** as a separable 2:1 mixture with **98** in 41 % yield. Total deprotection was achieved by a three-step protocol. The cyclohexylidene ketal first was converted to the corresponding diol with acetic

acid, then was subjected to deacetylation followed by hydrogenolysis over palladium hydroxide on carbon in aqueous dioxane in the presence of acetic acid. This sequence gave the 6'-epiaprosamine **19** as the acetate salt in 90% yield (5:1 ratio of anomers at the 8'-position) (Scheme 18).

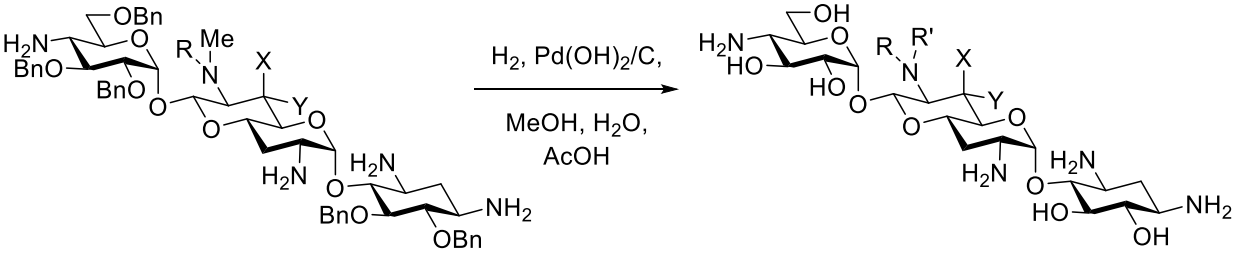
2.6.4. Unmasking of the apramycin derivatives

Global deprotection was typically accessed by a two-step protocol. Thus, all azido groups were first converted to the corresponding amines with trimethylphosphine and sodium hydroxide in hot aqueous THF (Staudinger reaction). Benzyl ethers were then removed by hydrogenolysis over palladium hydroxide[102] on carbon at atmospheric pressure in aqueous methanol in the presence of acetic acid giving the apramycin derivatives **107**-**115** and **117** (Scheme 19). Purification by Sephadex chromatography (CM Sephadex C-25), eluting with deionized water and then aqueous NH_4OH, afforded the apramycin derivatives in the form of the free bases. Finally, lyophilization in the presence of acetic acid gave the products in the form of their acetate salts for screening in the biological assays.



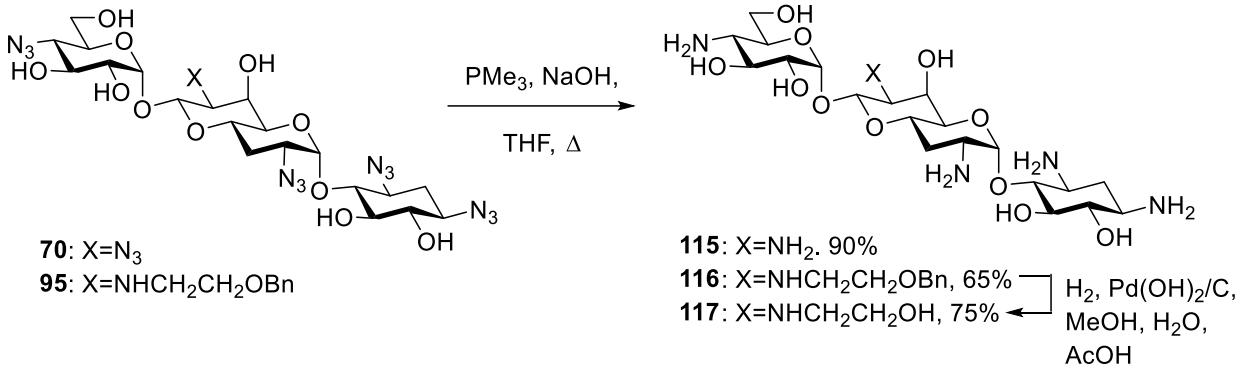
77: R=Cbz, R'=Me, X=H, Y=OH
78: R=Cbz, R'=Me, X=OH, Y=Me
86: R=Cbz, R'=Me, X=OH, Y=CF_3
87: R=Cbz, R'=Me, X=CF_3, Y=OH
88: R=Cbz, R'=Me, X=H, Y=N_3
90: R=Cbz, R'=Me, X=N_3, Y=H
93: R=Et, R'=H, X=OH, Y=H

100: R=Cbz, R'=Me, X=H, Y=OH, 85%
101: R=Cbz, R'=Me, X=OH, Y=Me, 70%
102: R=Cbz, R'=Me, X=OH, Y=CF_3, 90%
103: R=Cbz, R'=Me, X=CF_3, Y=OH, 80%
104: R=Cbz, R'=Me, X=H, Y=NH_2, 85%
105: R=Cbz, R'=Me, X=NH_2, Y=H, 76%
106: R=Et, R'=H, X=OH, Y=H, 71%



83: R=Cbz, R'=Me, X=Y=H
100: R=Cbz, R'=Me, X=H, Y=OH
101: R=Cbz, R'=Me, X=OH, Y=Me
102: R=Cbz, R'=Me, X=OH, Y=CF_3
103: R=Cbz, R'=Me, X=CF_3, Y=OH
104: R=Cbz, R'=Me, X=H, Y=NH_2
105: R=Cbz, R'=Me, X=NH_2, Y=H
106: R=Et, R'=H, X=OH, Y=H

107: R=X=Y=H, R'=Me, 55%
108: R=H, R'=Me, X=H, Y=OH, 60%
109: R=H, R'=Me, X=OH, Y=Me, 75%
110: R=H, R'=Me, X=OH, Y=CF_3, 60%
111: R=H, R'=Me, X=CF_3, Y=OH, 72%
112: R=H, R'=Me, X=H, Y=NH_2, 60%
113: R=H, R'=Me, X=NH_2, Y=H, 75%
114: R=Et, R'=H, X=OH, Y=H, 40%



70: X=N_3
95: X=$NHCH_2CH_2OBn$

115: X=NH_2. 90%
116: X=$NHCH_2CH_2OBn$, 65%
117: X=$NHCH_2CH_2OH$, 75%

Scheme 19: **Staudinger reaction and hydrogenolysis providing apramycin derivatives**

2.7. Biological evaluation

The above synthesized samples were submitted to the Böttger lab in Zurich, where they were screened for selectivity and antibacterial activity. As described in the Introduction the ribosomal drug susceptibility is studied by measurement of the IC_{50} against a single rRNA allelic derivative of the Gram-positive eubacterium *Mycobacerium smegmatis*.[103] Rabbit reticulocyte ribosomes were used as a source of authentic eukaryotic cytosolic ribosomes. Together with apramycin **6** and the 4,5-aminoglycoside antibiotics paromomycin **10** and neomycin B **14** as comparators all apramycin analogues were screened for their ability to inhibit ribosomal activity in the cell-free translation assays (Table 2).

Table 2: **Antiribosomal activities (IC50, µg/mL) and selectivities***

Compd	**Substitution Type**	**Bacterial activity**	**Mit13 Activity (Selectivity)**	**A1555G Activity (Selectivity)**	**Cyt14 Activity (Selectivity)**	**RRL**
6	apramycin	0.09	67.29 (747)	27.77 (308)	58.65 (652)	24.25 (269)
10	paromomycin	0.03	50.54 (1685)	5.83 (194)	10.39 (346)	9.78 (326)
14	neomycin B	0.01	1.62 (162)	0.22 (22)	17.12 (1712)	22.12 (2212)
107	6'-deoxy	>20	101.15	85.26	103.05	35.74
108	6'-epi-OH	0.74	124.21 (168)	45.08 (61)	90.01 (122)	66.34 (90)
109	6'-α-methyl	1.24	185.71 (150)	180.95 (146)	143.02 (115)	45.12 (36)
110	6'-α-CF_3	>20	>1000	>1000	>1000	n.d.

111	6'-epi-OH-6'-β-CF_3	>20	>543.33	>1000	>1000	n.d.
112	6'-deoxy-6'-α-amino	8.60	86.93 (10)	45.61 (5)	61.90 (7)	44.62 (5)
113	6'-deoxy-6'-β-amino	5.15	67.96 (13)	52.24 (10)	71.05 (14)	22.23 (4)
18	Aprosamine	1.99	56.69 (28)	23.35 (12)	49.19 (25)	36.08 (18)
19	6'-epiaprosamine	>10	104.21	36.51	104.25	72.33
115	7'-*N*-desmethyl	0.27	116.57 (431)	81.98 (303)	91.95 (340)	42.26 (156)
114	7'-*N*-ethyl	0.29	107.53 (371)	74.43 (256)	88.50 (305)	26.94 (93)
117	7'-*N*-(2-hydroxyethyl)	1.17	103.47 (88)	57.61 (49)	60.28 (51)	18.25 (15)

*Selectivities are obtained by dividing the eukaryotic activity by bacterial activity.

Complete removal of the hydroxyl group at the 6'-position showed significantly reduced activity of the AGA. When compared to parent apramycin **6**, the 6'-deoxy apramycin derivative **107** exhibits a >200 fold loss of activity. On the other hand the 6'-epiapramycin derivative **108** shows an approximately 10 fold loss of activity against bacterial wild-type ribosomes. Additionally, the deoxy compound **107** exhibits greater loss of activity (around 100 fold) as compared to the 6'-epi-compound **108** (3 to 5- fold loss) against the mitochondrial wild-type, the A1555G mitochondrial mutant, the cytosolic hybrid, and rabbit reticulocyte ribosomes (RRL). Thus, inversion of stereochemistry at the 6'-position of apramycin has only a small effect on ribosomal activity, whereas complete removal of the hydroxyl group has a very significant

negative effect on the interaction with ribosomes carrying the eukaryotic decoding A-site sequences. Thus, the results reveal the significance of the 6'-hydroxyl group and its configuration on the interaction of apramycin with the decoding A site. This is consistent with apramycin binding to the flipped-out conformation of the target decoding A-site with a hydrogen bonding pattern from the 6'-OH to N1 of A1408 as suggested by X-ray crystallographic studies using the complete 30S ribosomal subunit,[26] and in disagreement with other NMR and crystallographic studies using short models of the decoding A site in which the 6'-OH is exposed to water.[30] Incorporation of a methyl group at the 6'-position (**109**) or of a trifluoromethyl group in either configuration (**110** and **111**) is detrimental to antiribosomal activity in the bacterial wild-type and to a slight lower level in the eukaryotic hybrid ribosomes. These results also emphasize the greater influence of the 6'-hydroxy group and its configuration on binding to the bacterial rRNA A-site rather than to either of the eukaryotic ribosomes.

Further, the substitution of the 6'-hydroxyl group apramycin by an amino group is strongly detrimental to antiribosomal activity (**112** and **113**) and reduces the selectivity over the hybrid mutant ribosomes. This is presumably because protonation of one of the two amines in the vicinal diamine function precludes protonation of the second one,[104] and in doing so eliminates key interactions with the ribosome. For example, protonation of the 7'-amine would not allow protonation of the 6'-amine, which would greatly weaken the pseudobase pair interaction with A1408.

Removal of the 4-amino-4-deoxy-D-glucopyranose (ring III) from apramycin gave aprosamine **18**, which showed a ~20 fold loss of activity against bacterial wild-type ribosomes as well as hybrid mutants. On the other hand, 6'-epiaprosamine **19** also drastically lost activity against all tested ribosomes. This may be a consequence of the lack of hydrogen bonding

between C1409-G1491 base pair and the 5''O and 6''-OH positions of ring III. Overall, this situation is consistent with the pattern seen with apramycin and 6'-epiapramycin.

Simple removal of the methyl group from *N*7' (**115**), or its replacement by an ethyl group (**114**), results in a three-fold loss of inhibitory activity for all ribosomes. These modifications are affecting the bonding character between *N*7' and phosphate back bone of A1492, A1493 bases of bacterial rRNA. Finally, the replacement of methyl group by hydroxyethyl group **117** reduced the activity against the wild-type bacterial ribosomes.

Table 3: **Compound interaction with polymorphic residues in the drug binding pocket (IC_{50}, µg/ml)**

		Bacterial A Site			
Compd	**6'-Substitution Type**	**Wild Type**	**G1491C**	**G1491A**	**A1408G**
6	apramycin	0.09	31.21 (347)	5.00 (55)	128.9 (1425)
10	paromomycin	0.03	10.42 (347)	0.57 (19)	0.26 (9)
14	neomycin B	0.01	0.67 (67)	0.06 (6)	17.51 (1751)
108	6'-epi-OH	0.74	89.66 (121)	16.01(21)	86.88 (117)
115	7'-*N*-desmethyl	0.27	152.90 (566)	44.86 (166)	133.86 (496)
114	7'-*N*-desmethyl-7'-*N*-ethyl	0.29	117.16 (403)	21.56 (73)	189.50 (653)

*Selectivities are obtained by dividing the single mutant activity by bacterial activity.

In an attempt to gain further understanding, the antiribosomal activity of the better compounds **108**, **114** and **115** towards bacterial ribosomes carrying single point mutations in the decoding A site was determined. The study is focused on the comparison with parent compound apramycin (**6**), and the comparators paromomycin (**10**), and neomycin B (**14)** and their ability to inhibit the bacterial wild-type ribosome in contrast to its A1408G, G1491C and G1491A mutants (Table 3).

From this study, it is clear that the apramycin and neomycin B exhibit superior selectivity for the wild-type over the A1408G mutant than 6'-*epi*-apramycin and the other compounds. The reason could be the outcome of the imposed *gg*-conformation of the apramycin C5'-C6' bond disfavouring pseudo-base pair formation of the AGA O5' and 6'-OH groups with *N*1 and *N*2 of G1408.[105,106] When compared with the 1408-ring I interaction, the 1491-ring I interaction is probably less susceptible to inversion of configuration at the apramycin 6'-position. The relocation of the axial 6'-hydroxyl group from the β-face of the bicyclic apramycin ring I to the equatorial position on the α-face (6'-epiapramycin) similarly affects the drug binding pockets both containing the C1491=G1409 Watson-Crick pair and mutants with C1491●C1409 and A1491●C1409 non-canonical base pairs.

Table 4: **Minimal inhibitory concentrations (MIC, µg/ml) of clinical isolates**

Compd	**Strain**							
	***Staphylococcus aureus* (MRSA)**					***Escherichia coli***		
	AG 038	**AG 039**	**AG 042**	**AG 044**		**AG 001**	**AG 055**	**AG 003**
6	8	8	8	16		16	8	8-16
10	4	>128	>128	4-8		16-32	8	8-16
14	0.5-1	nd	128	0.5-1		8-16	nd	4
107	>128	>128	>128	>128		>128	>128	>128
108	32-64	64	64	32-64		32	32	32
109	≥64	≥64	≥64	≥64		32-64	32-64	32-64
110	>64	>64	>64	>64		>64	>64	>64
111	>64	>64	>64	>64		>64	>64	>64
112	>128	>128	>128	>128		>128	128	128

113	>128	>128	>128	>128		>128	>128	>128
18	32-64	32-64	32	32		64-128	64-128	32-64
19	>128	>128	>128	>128		>128	>128	>128
115	16-32	32	16-32	16		16-32	16-32	16
114	16	8-16	16	8-16		8-16	8-16	8-16
117	64	≥64	64	≥64		32-64	32-64	32-64

All apramycin derivatives prepared, together with the parent apramycin **6** and other comparators **10**, and **14**, were screened for antibacterial activity against clinical isolates of *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* (Table 4). Most synthetic apramycin derivatives exhibit significantly reduced activity compared to parent apramycin and the comparators **10** and **14**, which was anticipated on the basis of their cell-free ribosomal translation assays data (Tables 2.2). The 6'-epiapramycin **108** showed moderate to good activity against some strains of *S. aureus* and/or *E. coli*, whereas 6-α-methylapramycin **109** was moderately active only against *E. coli*. Aprosamine **18** exhibits moderate activity and 6'-epiaprosamine **19** completely lost activity against all strains studied. The 7'-*N*-desmethyl apramycin derivative **113** and the 7'-*N*-desmethyl-7'-*N*-ethyl derivative **114** have moderate to good activity, in some cases comparable with apramycin, against all strains tested. The 7'-*N*-hydroxyethyl derivative **117** is less effective against all strains, as compared to substitution by a simple ethyl group **114**.

Overall, the inversion of the stereochemistry of 6'-hydroxy group of apramycin lessens the antiribosomal and antibacterial activity of this AGA; while deletion of the 6'-hydroxy group substantially diminishes all activity. Also, the replacement of the 6'-hydroxy group by an amino group is detrimental to activity. The overall results point to changes at the 6'-position of

apramycin having a greater influence on binding to the wild-type bacterial ribosomes than drug binding pockets of the eukaryotic ribosomes. The results are consistent with apramycin adopting the standard binding mode of the 4,5- and 4,6-aminoglycosides in the decoding A site of the bacterial ribosome as opposed to the alternative binding mode proposed in some studies.

2.8. Conclusion

A series of aminoglycoside antibiotic apramycin derivatives have been prepared by modifying the 6'- and *N*7'-positions and were screened for antiribosomal activity in cell-free translation assays with a series of wild-type and mutant ribosomes, as well as for antibacterial activity against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* (E coli). All apramycin derivatives prepared showed a greater loss of activity against the bacterial wild-type ribosome than against the hybrid mutants of the human mitochondrial and cytosolic ribosomes. The existence and its exact stereochemical location of the hydroxyl group at the 6'-position is more critical for binding to the bacterial decoding A site than to either of its mitochondrial or cytosolic ribosomes. These studies about activity changes between the various ribosomes contribute to the current understanding of the mode of interaction of AGAs with the bacterial ribosomsal decoding A site and should support future design and development of more active and less toxic aminoglycoside antibiotics.

CHAPTER 3. SYNTHESIS AND BIOLOGICAL EVALUATION OF PAROMOMYCIN ANTIBIOTICS CARRYING AN APRAMYCIN-LIKE RING I

3.1. Introduction

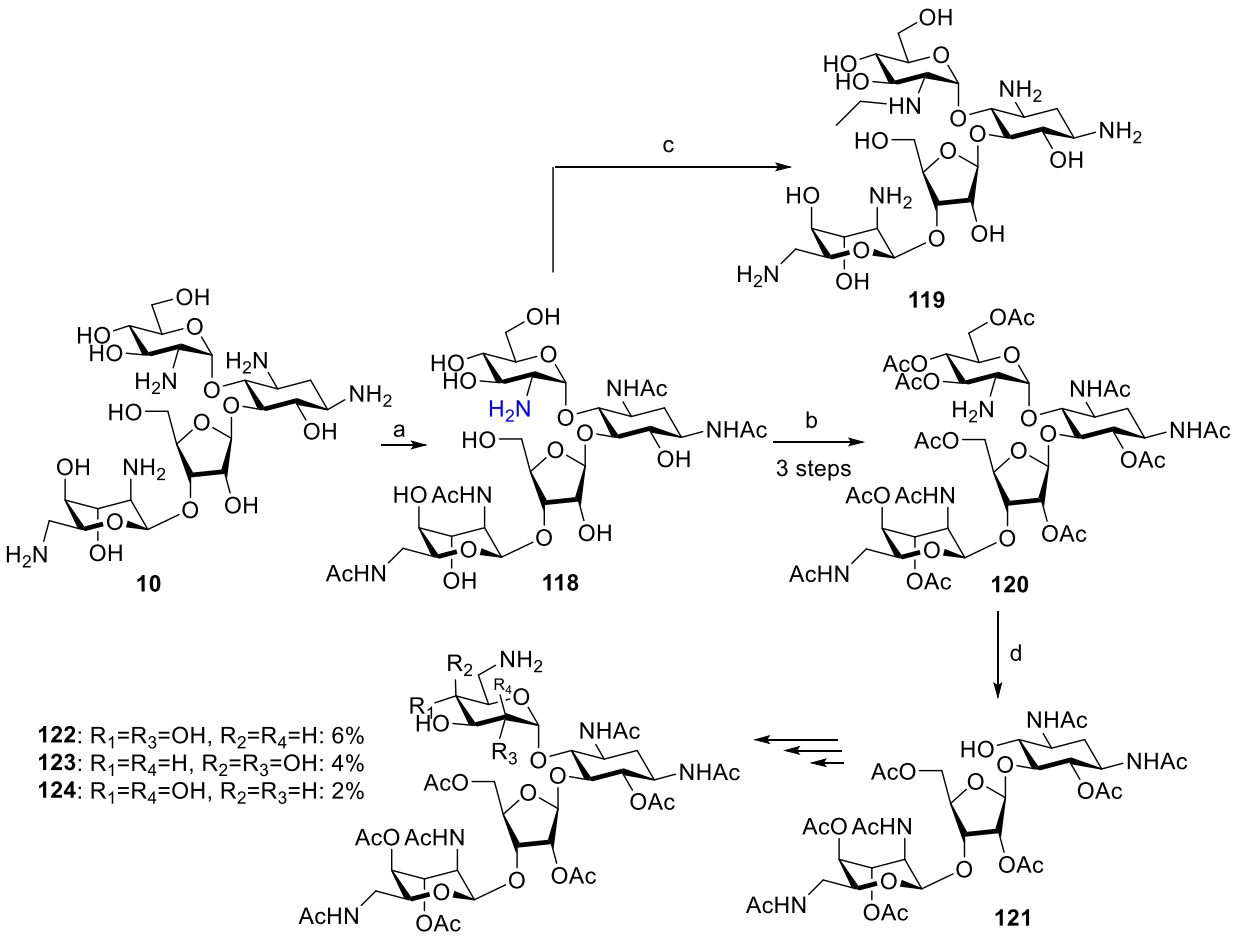
Paromomycin is a representative of the 4,5-disubstituted 2-deoxystreptamine aminoglycoside antibiotics. It is an effective antibiotic against Gram-negative and several Gram-positive bacteria but is no longer used in North America as an antibiotic due to its poor therapeutic index. Paromomycin is licensed as an effective, well tolerated treatment for visceral leishmaniasis (*kala-azar*) in India.[107] This chapter describes the synthesis of paromomycin analogues carrying an apramycin-like scaffold in place of ring I and the influence of these modifications on antiribosomal and antibacterial activity.

3.2. Existing modifications of paromomycin ring I

3.2.1. Modification of the 2',3' & 4'-positions of paromomycin

Researchers have invested considerable effort towards generating new paromomycin analogues by modifying its key functional groups. Modifications of ring I of paromomycin to produce the new antibiotics are surveyed here. The modification of ring I of paromomycin was first attempted with the synthesis of 2'-*N*-ethylparomomycin in the 1980s. The key intermediate 1,3,2''',6'''-tetra-*N*-acetylparomomycin **118** was obtained from paromomycin **10** using acetic anhydride in aqueous methanol. Subsequently, reductive alkylation of **118** with acetaldehyde and sodium borohydride followed by deacetylation afforded 2'-*N*-ethylparomomycin **119**. This compound showed comparable activity with the parent paromomycin against selected microorganisms.[108] Carbamate protection of 1,3,2''',6'''-tetra-*N*-acetylparomomycin **118** at the *N*2'position, per-acetylation followed by hydrogenolysis of the benzyloxycarbonyl afforded the 1,3,2''',6'''-tetra-*N*-acetyl-octa-*O*-acetyl-paromomycin **120**. Deamination of **120** with nitrous acid gave pseudotrisaccharide **121**, which was used for the replacement of ring I of paromomycin

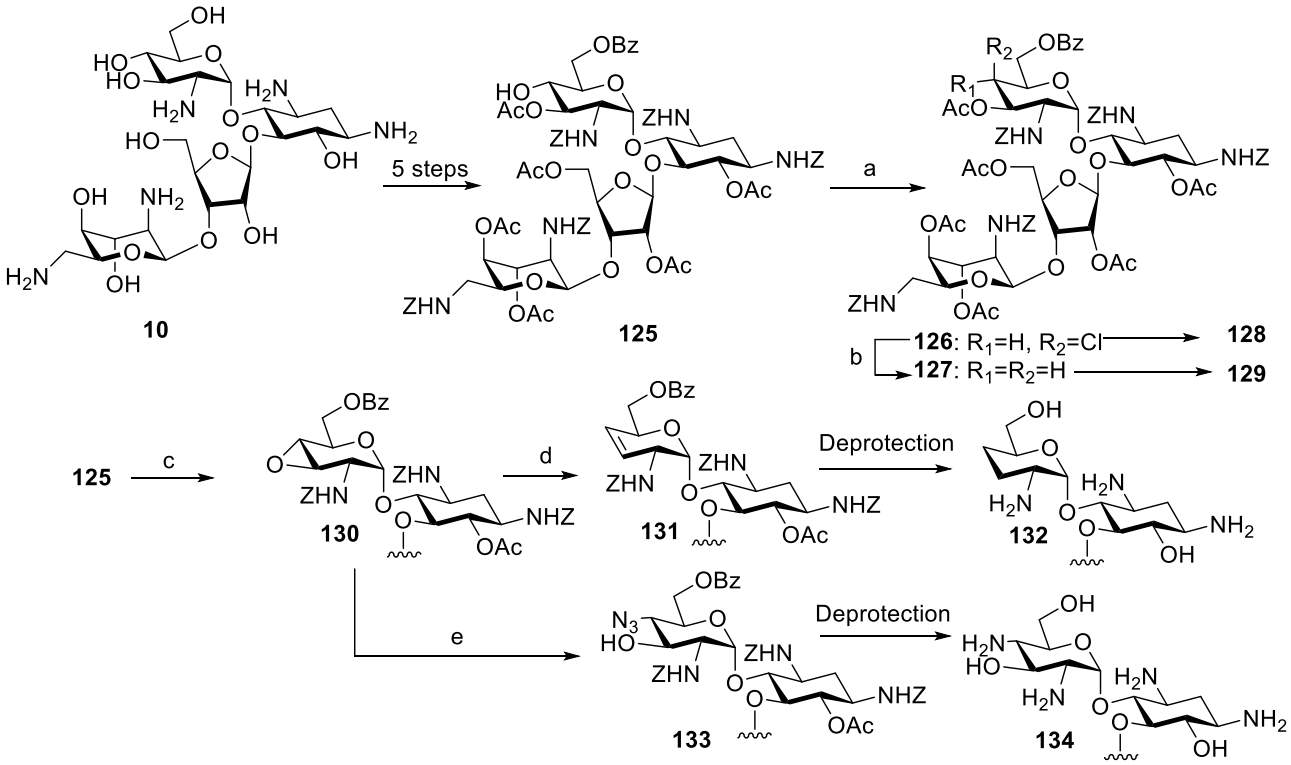
with various glycosides (Scheme 20).[109] Among these ring I analogues of paromomycin, only the 6'-amino-6'-deoxy-glucopyranosyl derivative **122** exhibited slightly reduced activity; the galacto- (**123**) and manno- (**124**) derivatives had dramatically lower activity when compared with paromomycin.[109]



***Scheme 20*: Synthesis of 2'-*N*-ethylparomomycin and ring I analogues of paromomycin**

Modification of the 3' and 4'-positions of paromomycin involved a 5 step sequence to achieve 4'-hydroxyl intermediate **125** starting with the carbamate protection of all amines followed by benzylidene protection of the 4',6'-diol. After that, the remaining hydroxyl groups were protected as acetates and cleavage of the benzylidene ring followed by selective 6-*O*-

benzoylation gave the 4'-hydroxyl intermediate **125**.[110] Reaction of **125** with sulfuryl chloride provided the 4'-epichloro-4-deoxy derivative **126**, which gave 4'-deoxyparomomycin derivative **127** by reductive removal of the halogen using tributyltin hydride (Scheme 21).



a) SO_2Cl_2, 51%: b) Bu_3SnH, AIBN, 90%: c) MsCl; NaOMe, 60%: d) NaI; MsCl, Pyridine, 33%: e) NaN_3, 40%

***Scheme 21*: Synthesis of 3',4' modified paromomycin derivatives**

O-Mesylation of compound **125** at the 4'-position and subsequent treatment with sodium methoxide provided the 3',4'-β-epoxide **130.** The reaction of **130** with sodium iodide in acetone afforded an iodohydrin which on treatment with methanesulfonyl chloride in pyridine gave the unsaturated paromomycin derivative **131**. Similarly, the reaction of **130** with sodium azide lead to the 4'-azido-4'-deoxy intermediate **133**. All these intermediates were subjected to conventional deprotection methods to give the corresponding paromomycin derivatives (Scheme 21). Among these molecules the 4'-deoxy **129** and 3',4'-dideoxy **132** paromomycin derivatives exhibited

slightly improved activity against a number of bacterial strains, while the deoxy amine **134,** showed comparable activity in ribosomal assays.[110,111] In contrast Vasella and coworkers later reported that the 4'-amino-4'-deoxy derivative **134** and the 4'-deoxy derivative of paromomycin **129** show slightly less activity than the parent antibiotic. These workers also confirmed that the galacto configured ring I derivatives **135** & **136** exhibit lesser activity than the corresponding gluco-configured analogues (Figure 19).[112]

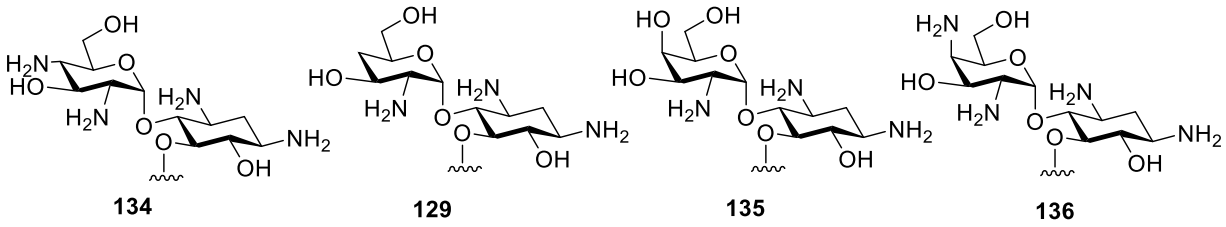
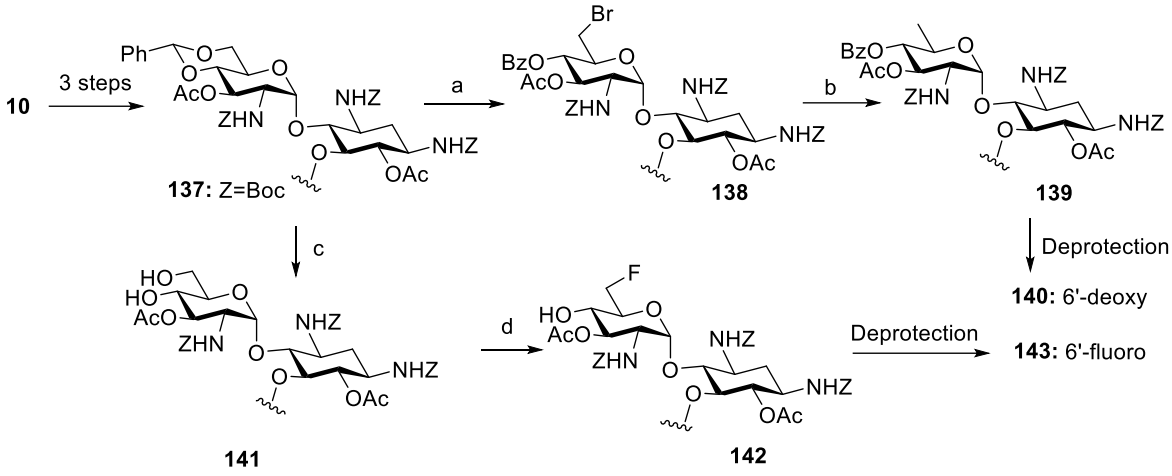


Figure 19: **Some 4'-modified paromomycin derivatives**

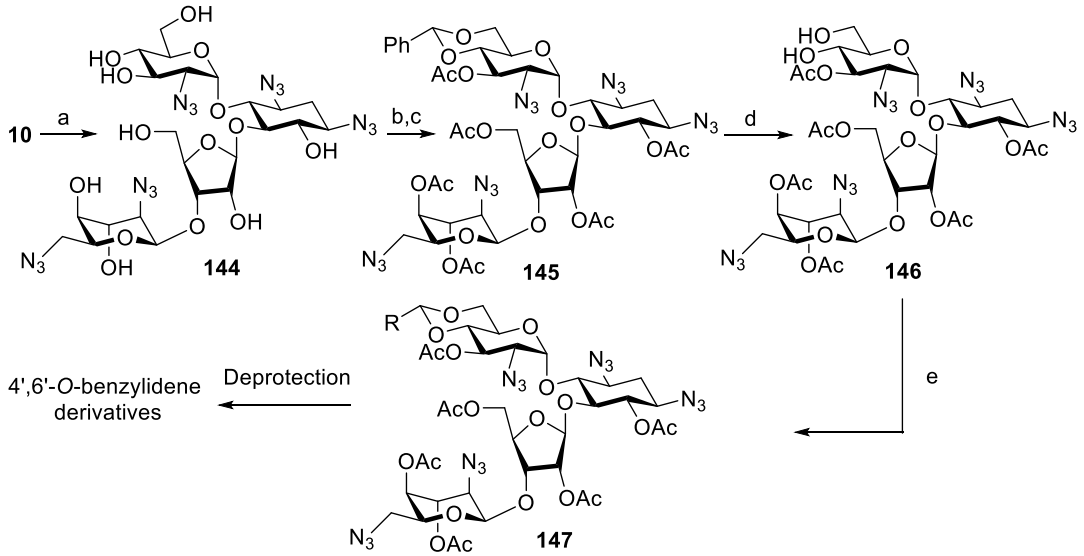
3.2.2. Modification of the 4' & 6'-positions of paromomycin

In recent years, researchers have focused on modification of the 4' and 6'-positions of paromomycin. Various crystallographic studies and existing literature on paromomycin revealed the role of the 6'-hydroxyl group in binding to the bacterial ribosomal RNA. Consequently Vasella and coworkers investigated the importance of the 6'-hydroxyl group by replacing it with fluorine and by complete exclusion of hydroxyl group as shown in Scheme 4. Regioselective ring opening of the benzylidene ring of **137** followed by radical dehalogenation afforded the 6'-deoxyparomomycin **139**. After that, treatment of **137** with aq. acetic acid gave the 4',6'-diol **141**, which was subjected to the fluorination of the 6'-hydroxyl group using DAST giving 6'-fluoro-6'-deoxyparomomycin **142**. Deacetylation followed by removal of the Boc groups gave 6'-deoxy **140**, and 6'-fluoro-6'-deoxyparomomycin **143**, which were 16 to 32 times less active than the original paromomycin (Scheme 22).[113]



a) $BaCO_3$, NBS, CCl_4, 80 °C, 55% : b) Bu_3SnH, AIBN,Toluene, 80 °C, 72%: c) 80% AcOH, 68%: d) DAST, -55 °C, 67%

Scheme 22: **Synthesis of some 6'-paromomycin derivatives**

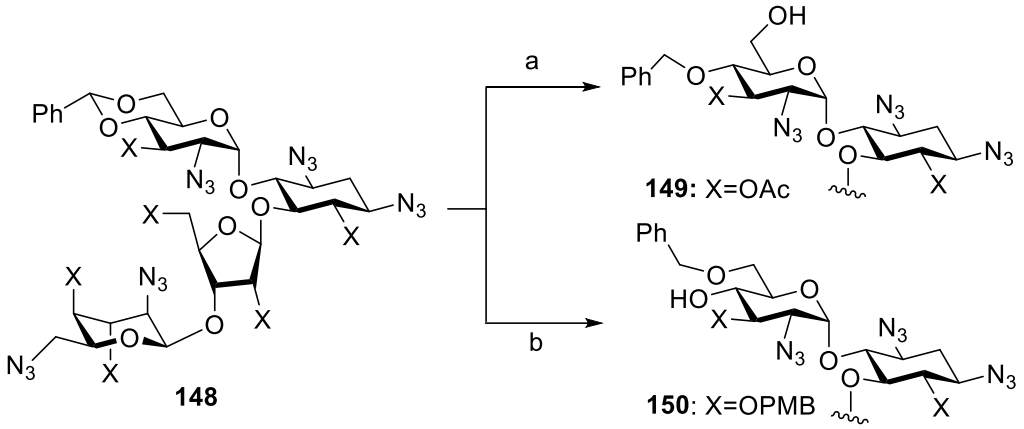


a) TfN_3, $CuSO_4$, *t*-BuOK, *t*-BuOH, H_2O, 49%: b) PhCHO, HCOOH, O °C, 75%: c) Ac_2O, Py, DMAP, 75%: d) $TsOH.H_2O$, MeOH, 61%: e) RCHO, $FeCl_3$, MeOH, 61% or RCHO, $TsOH.H_2O$

Scheme 23: **Synthesis of 4',6'-*O*-benzylidene derivatives of paromomycin**

In the European patent 1,953,171 the synthesis of paromomycin derivatives altered at the 4',6'-position with a range of substituents was reported together with their antibacterial activity.[114] The synthesis of 4',6'-*O*-benzylidene substituted paromomycin antibiotics can

achieved in six synthetic steps. Paromomycin was subjected to the copper-catalyzed diazo transfer reaction using triflyl azide to give penta azide **144.** Subsequently, benzylidenation followed by acetylation of remaining hydroxyl groups provided the fully protected intermediate **145**. Cleavage of the 1, 3-dioxanyl ring of **145** with PTSA gave the 4',6'-diol intermediate **146**. Then, the reaction of **146** with a range of aromatic aldehydes or acetals gave the desired 4',6'-*O*-benzylidenated intermediates. Finally, the unmasked 4', 6'-benzylidenated paromomycin compounds were achieved after deprotection (Scheme 23).[114]

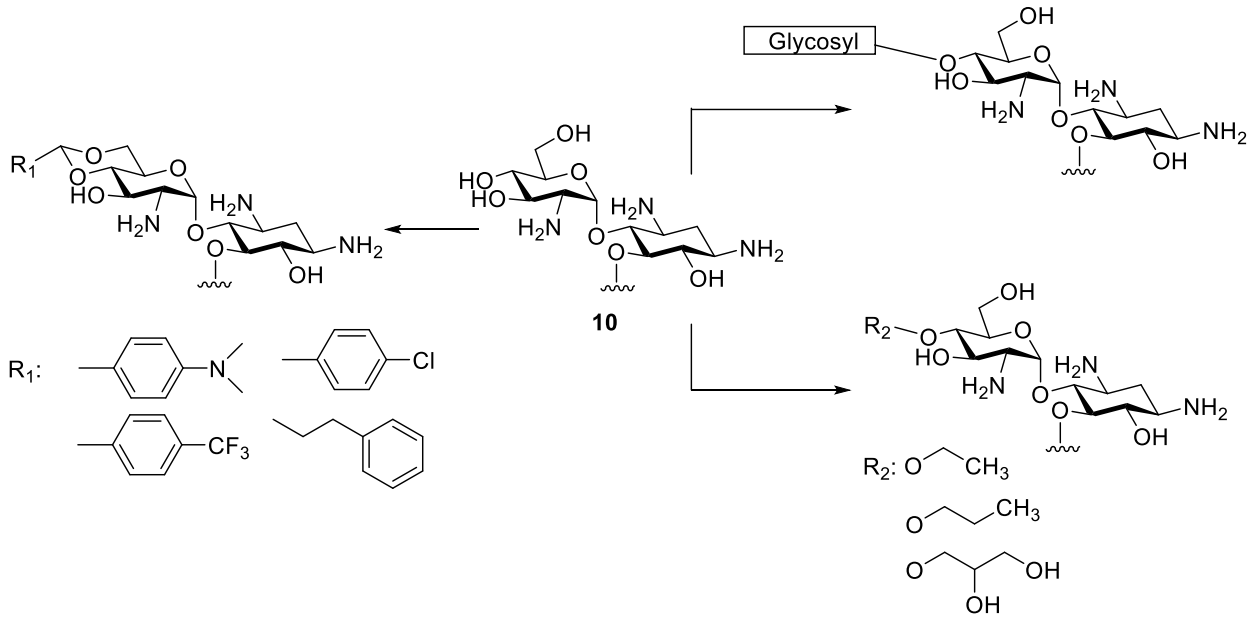


a) $BH_3.Me_2S$ in THF, Bu_2BOTf in DCM, DCM, 61%: b) NaH, p-MeOBnCl, Bu_4NI, THF, 48%; $NaCNBH_3$, 0.7 M HCl in Et_2O, O °C, 33%

Scheme 24: **Regioselective opening of the 1, 3-dioxanyl ring of 148**

Furthermore, Vasella and coworkers reported the regioselective opening of the 1,3-dioxanyl ring of **148** under two sets of conditions to give regioisomeric products (Scheme 24). This approach is useful for selective modification at the 4' and 6'-positions of paromomycin. Using these two strategies, a library of paromomycin derivatives including the 4'-*O*-alkyl, 4'-*O*-aralkyl and the 4',6'-acetals[24,114,115] were synthesized and their ribosomal activity profile was reported. These molecules retained their activity against a range of clinical isolates and showed improved selectivity in evasion of mitochondrial and cytoplasmic ribosomes when compared to

the parent paromomycin. Recently, Crich group has explored the 4'-position of paromomycin further by adding an additional ring using glycosylation. Thus, a range of 4'-*O*-glycosyl paromomycin analogs and a 4'-*O*-(glucosyloxymethyl) derivative were synthesized and the influence of the glycosyl moiety on their protein synthesis inhibitory action by bacterial, mitochondrial and cytosolic ribosomes was studied (Scheme 25).[116,117]



Scheme 25: **Various modifications at the 4' and 6'-positions of paromomycin**

Overall, several strategies have been reported to synthesize new paromomycin analogues by modification of ring I with emphasis on the 4'-position or both the 4'- and 6'-positions. Modifications of interest include 4'-*O*-alkyl chains, 4',6'-*O*-acetals as they displayed interesting biological profiles in terms of activity and selectivity.

3.3. Design of new paromomycin antibiotics

According to the existing data, rings I and II of the 4,5- and 4,6-disubstituted 2-deoxystreptamine class of aminoglycosides are mostly accountable for drug binding to the bacterial 30S ribosomal A site. It is established that the glucopyranosyl ring (ring I) of these AGAs takes part in a pseudo-base pair interaction with the A1408 nucleotide. In particular, N1

and N6 of A1408 base form hydrogen bonds with the 6'-substituent (OH in paromomycin **10** or NH_2 in neomycin **14**) and the ring I oxygen (O5') of the AGA,[12,24,26,118] as described in detail in chapter 1, section 1.42. In these type I interactions complexes the glucopyranosyl side chain consistently adopts the *gauche*, *trans* (*gt*) conformation[119] (Figure 20B). This is to be contrasted with the apramycin ring I-A1408 interaction,[120,121] where the 6'-hydroxy group is locked in the *gauche*, *gauche* (*gg*) conformation leading to a type II interaction with the ribosome (Figure 20B).

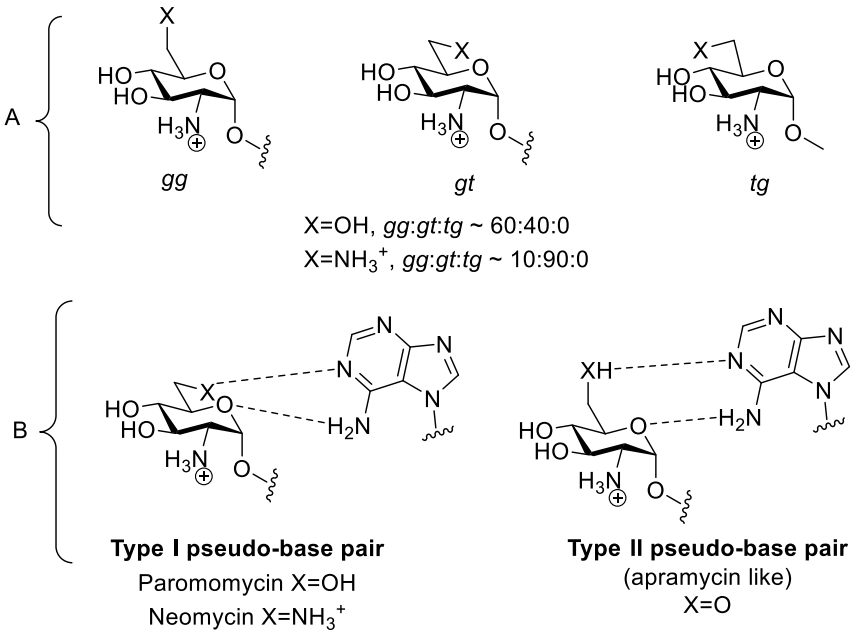


Figure 20: **A) Side chain conformations of ring I and estimated populations based on methyl α-D-glucopyranoside and methyl 6-amino-6-deoxy-α-D-glucopyranoside, B) Type I and II Pseudo-base Pairs**

Additionally, the existing literature of 4'-*O*-alkyl paromomcyin derivatives shows that an alkyl chain length of 2 or 3 carbons is ideal to afford the optimum reduction in mitoribosomal activity with the minimum loss of anibacterioribosomal activity. In the 4',6'-*O*-alkylidene paromomycin derivatives the ethylidene derivative **153** had a better activity profile than the analogous methylidene derivative **151**, which also suggests a 2 carbon alkyl chain on O4' to be

optimal.[115] Furthermore, it is known that the unusual monosubstituted 2-deoxystreptamine AGA apramycin **6** is not ototoxic in animal models consistent with predictions, based on cell free translation assays of mitochondrial ribosomes.[25] Taking all of these factors into consideration paromomycin-apramycin hybrids were designed to improve the antibacterial activity and reduce the toxicity profile of paromomycin.

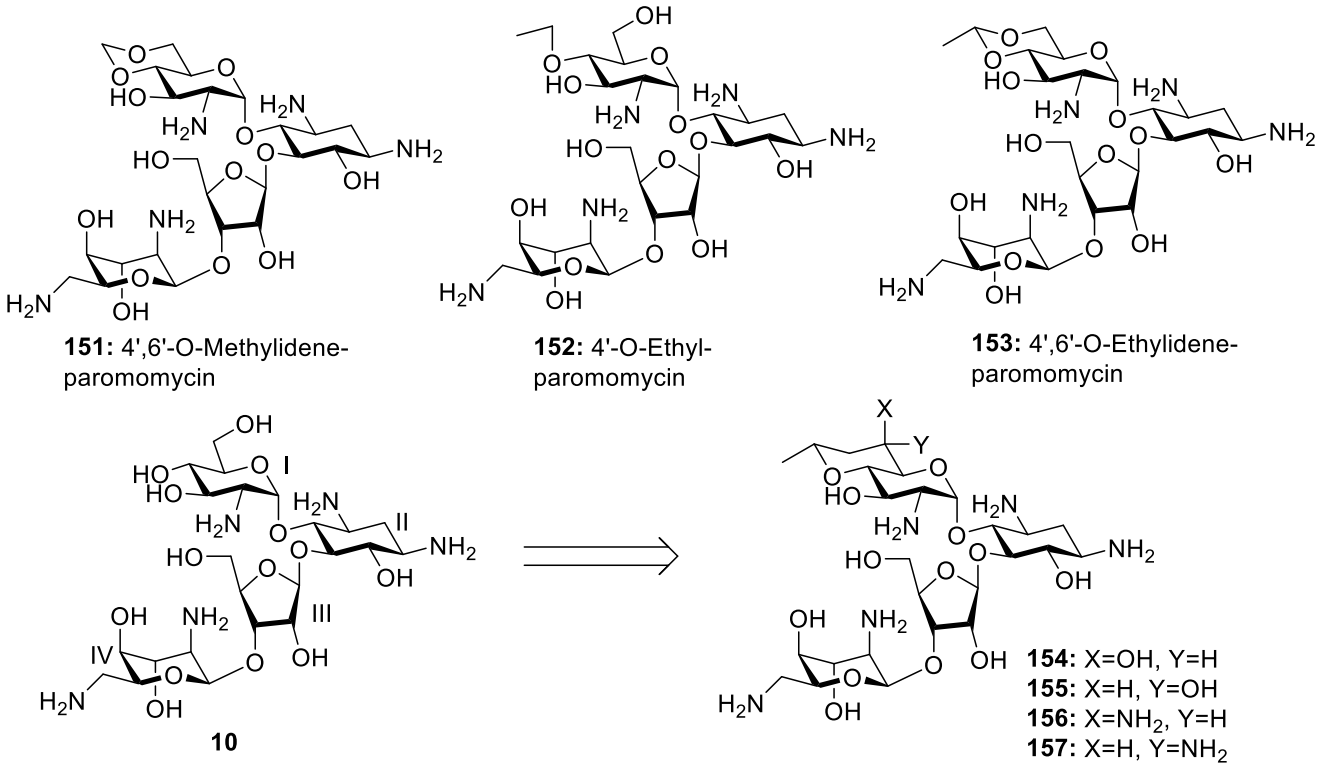


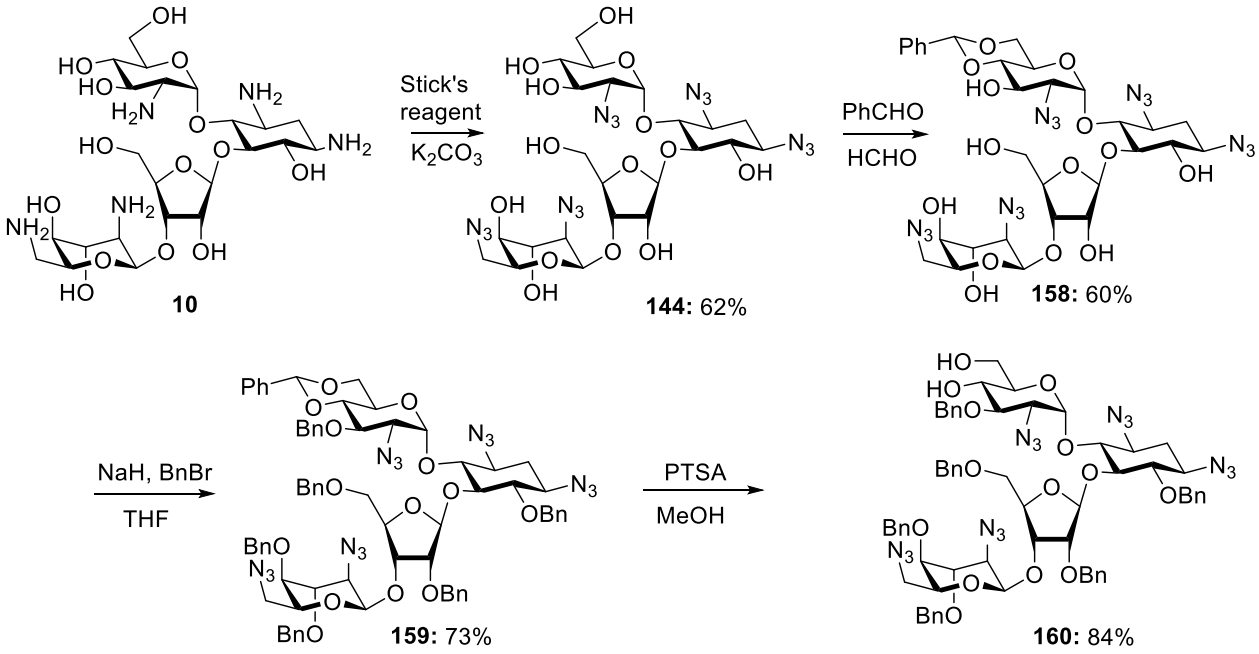
***Figure 21*: Design of the new class of paromomycin analogs**

The designed paromomycin-apramycin hybrids consist of paromomycin derivatives in a *trans*-dioxadecalin-like structure replaces ring I of the parent. The new bicyclic ring carries a methyl substituent placed so as to resemble the ethylidene acetal **153**. The bicyclic ring also carries either an equatorial or axial amine located in such a way as to participate in a type I or type II pseudobase interaction with A1408 (Figure 21). The following sections discuss work

conducted to synthesize the target molecules (**153-157**) and their effect on antiribosomal and antibacterial activity.

3.4. Results and discussion

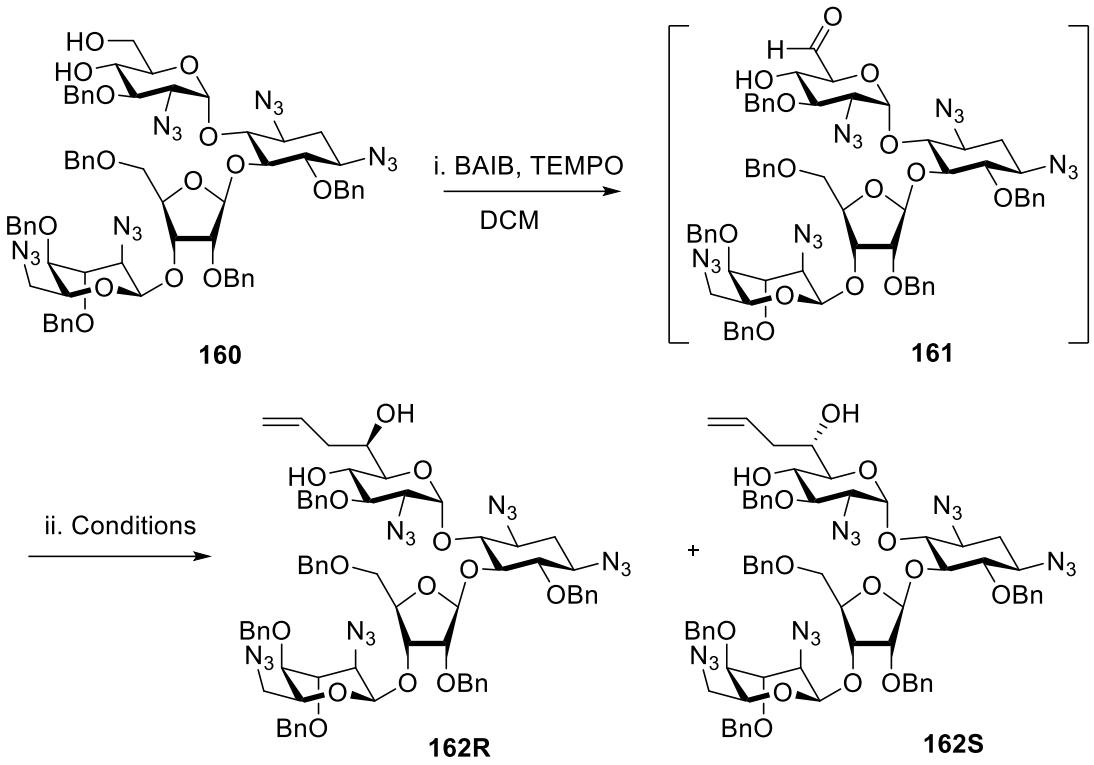
3.4.1. Synthesis of a paromomycin 4',6'-diol intermediate



Scheme 26: **Synthesis of a protected paromomycin 4',6'-diol derivative**

The synthesis began by diazo transfer to paromomycin sulfate **10** with imidazole sulfonyl azide (Stick's reagent)[87] which afforded the known 1,3,2',2''',6'''-pentaazido derivative **144** in 62% yield. Subsequent acid catalyzed benzylidene protection of **144** at the 4' and 6' hydroxyl groups gave **158** in 60% yield. Benzylation of all the remaining hydroxyl groups using benzyl bromide in the presence of sodium hydride gave **159** (73%) and was followed by the cleavage of benzylidene ring, which was achieved by reaction with *p*-toluenesulfonic acid in methanol affording the common intermediate **160** in 84% yield. This intermediate allows the subsequent selective facile modifications at the 4'- and 6'-positions (Scheme 26).

3.4.2. Synthesis of a 6'-allylparomomycin derivative



Scheme 27: **Synthesis of a 6'-allylparomomycin derivative**

The diol **160** was subjected to selective oxidation using TEMPO and BAIB in DCM,[122] and, without purification, the resultant aldehyde was subjected to the allylation (Scheme 27). Numerous conditions for allylation were tried leading to the results presented Table 5.

Table 5: **Allyllation of aldehyde 161**

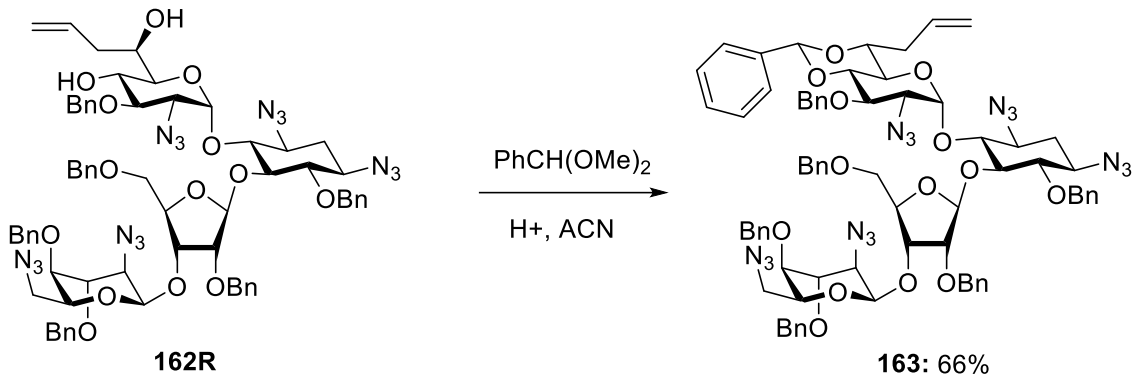
Entry	Conditions	Remarks [162*R* & 162*S*-isomers]
1	Allyltributyltin BF_3OEt_2, DCM[123]	30%, 28% (1:1 ratio)
2	(+)-Ipc_2Allyl/Diethylether, 3N NaOH/30% H_2O_2[124]	200 mg Scale: 40% & 3% (1:0.1) 2.0 g Scale: 25% & 28% (1:1)

3	AllylTMS, $TiCl_4$, DCM[125]	1:1.3 ratio*
4	AllylMgBr in Et_2O[125]	Starting material decomposed

*Not isolated

First, aldehyde **161** was treated with allyltributyltin and boron trifluoride diethyl etherate in DCM which gave the 6'-allylated derivative **162*R*** in 30% yield and the *S*-isomer **162*S*** in 28% yield, whose configurations were determined as described below. To improve diastereoselectivity of the allylation reaction Brown allylation was attempted. Thus , B-allyldiisopinocampheylborane was synthesized from (-)-α-pinene in three steps according to the literature protocol.[124] On a smaller scale the use of this reagent gave a percentage of the desired R-alcohol, but upon the increasing the scale of the reaction inconsistent results were observed. Allylation with allyltrimethylsilane in the presence of titanium tetrachloride also gave 1:1.3 ratio of R and S-alcohols. On the basis of this brief survey, allyltributyltin was selected as the preferred reagent.

The absolute configuration of the newly formed ring in compound **162*R*** was confirmed by conversion to its benzylidene derivative **163** by treatment with benzaldehyde dimethylacetal in the presence of catalytic amount acid in acetonitrile. The nOe spectrum of **163** showed clear enhancement of the resonances for H-4' and H-6' on irradiation of the benzylidene proton (Scheme 28).



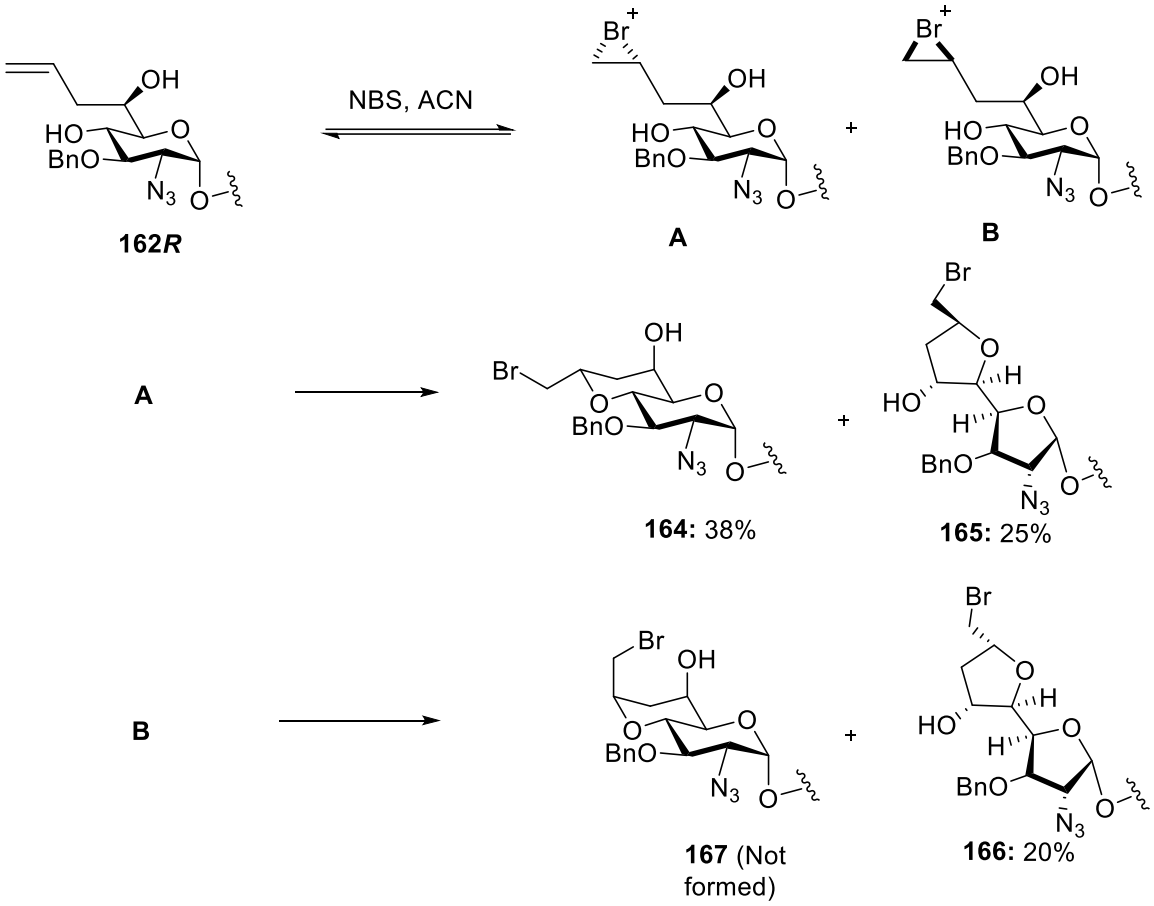
Scheme 28: **Synthesis of 4',6'-*O*-benzylidene derivative of 162*R***

3.4.3. Synthesis of a bicyclic ring I for paromomycin

The 6'-allylparomomycin derivative **162*R*** was subjected to bromocycloetherification using *N*-bromosuccinimide in acetonitrile to give the cyclized product **164** in 38% yield along with two furanosyl derivatives **165** and **166** in 20% and 22% yields, respectively (Scheme 29). The axial location of the alcohol in **164** confirmed the assignment of configuration of the substrate. The relative configurations of the two furanosyl products were assigned following complete deprotection. The mechanism of formation of **164**-**166** involves the reversible formation of two diastereomeric cyclic bromonium ions A and B, which are in equilibrium with the substrate[126,127] as shown in Scheme 10. The formation of cyclic product **164** involves the attack of the 4'-hydroxyl group on cyclic bromonium ion A in a 6-exo fashion. The formation of **165** and **166** involves the participation of the ring oxygen to open the cyclic bromonium ions A and B in a 5-exo approach, respectively. The cyclized product **167**, arising from attack of the 4'-hydroxy group on bromonium ion B, was not formed probably due to severe 1,3-diaxial repulsions between the hydroxyl group and bromomethyl group.

Bromocycloetherification of the diastereomeric homoallyl alcohol **162*S*** gave only furanosyl derivatives. The contrast in results between diastereomers **162*R*** and **162*S*** results can

be explained by analysis of the respective side chain conformations. In case of isomer **162*R***, both the *gg* and the *gt* conformations are expected to be populated and leading to the bicyclic **164** and the two furanosides **165** and **166**, respectively. With isomer **162*S*** the preferred conformation will be the *gg*, which will lead to furanoside products (Figure 22).



Scheme 29: **Synthesis of ring I modified cyclic derivatives of paromomycin**

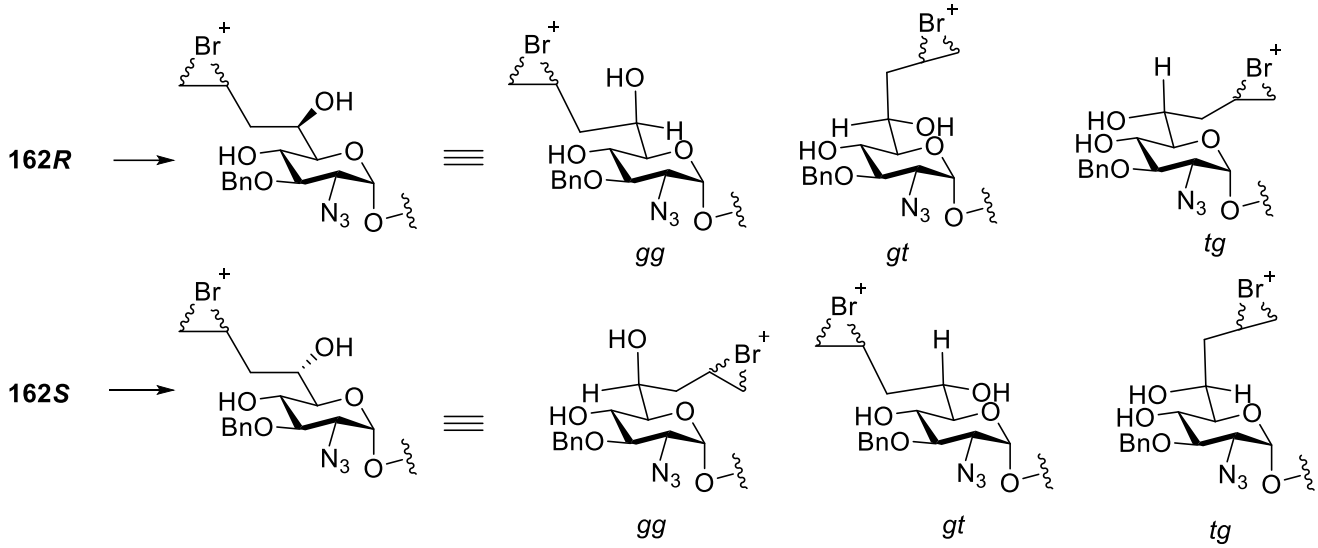
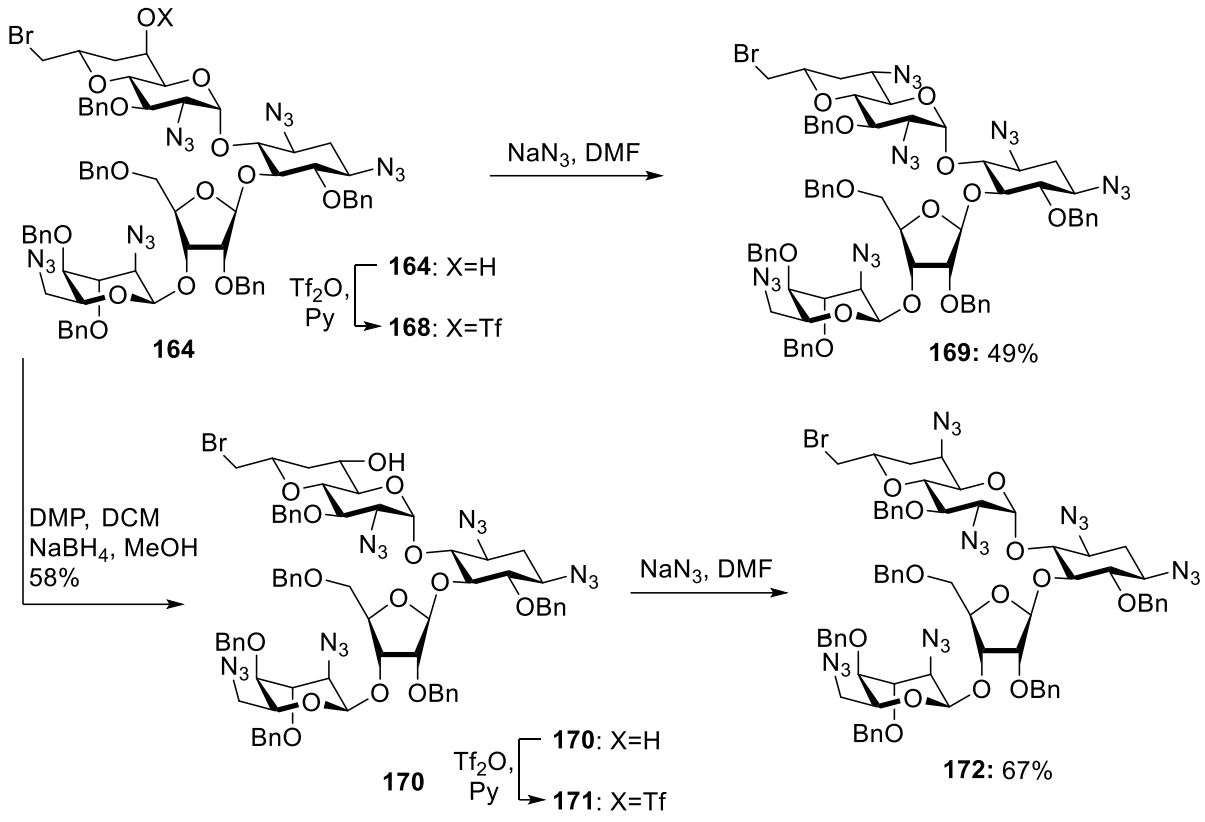


Figure 22: **Side chain conformations of ring I of 162*R* and 162*S***

3.4.4. Derivatization of the 6'-position of bicyclic paromomycin

Bicyclic compound **164** was subjected to the oxidation with Dess Martin periodinane[90] reagent which afforded the 6'-ketone. This was further subjected to the reduction with sodium borohydride in methanol to give the bicyclic 6'-equatorial hydroxy paromomycin derivative **170** as a separable 3:1 mixture with **164** in 58% yield. The bicyclic 6'-equatorial azido paromomycin derivative **169** was accessed from the corresponding inverted 6'-triflate **168** in 49% yield by displacement with sodium azide in DMF. The bicyclic 6'-axial azido paromomycin derivative **172** was achieved from the corresponding inverted 6'-triflate **171** in 67% yield, by displacement with sodium azide in DMF (Scheme 30).

3.4.5. Deprotection of the paromomycin-apramycin hybrid analogues



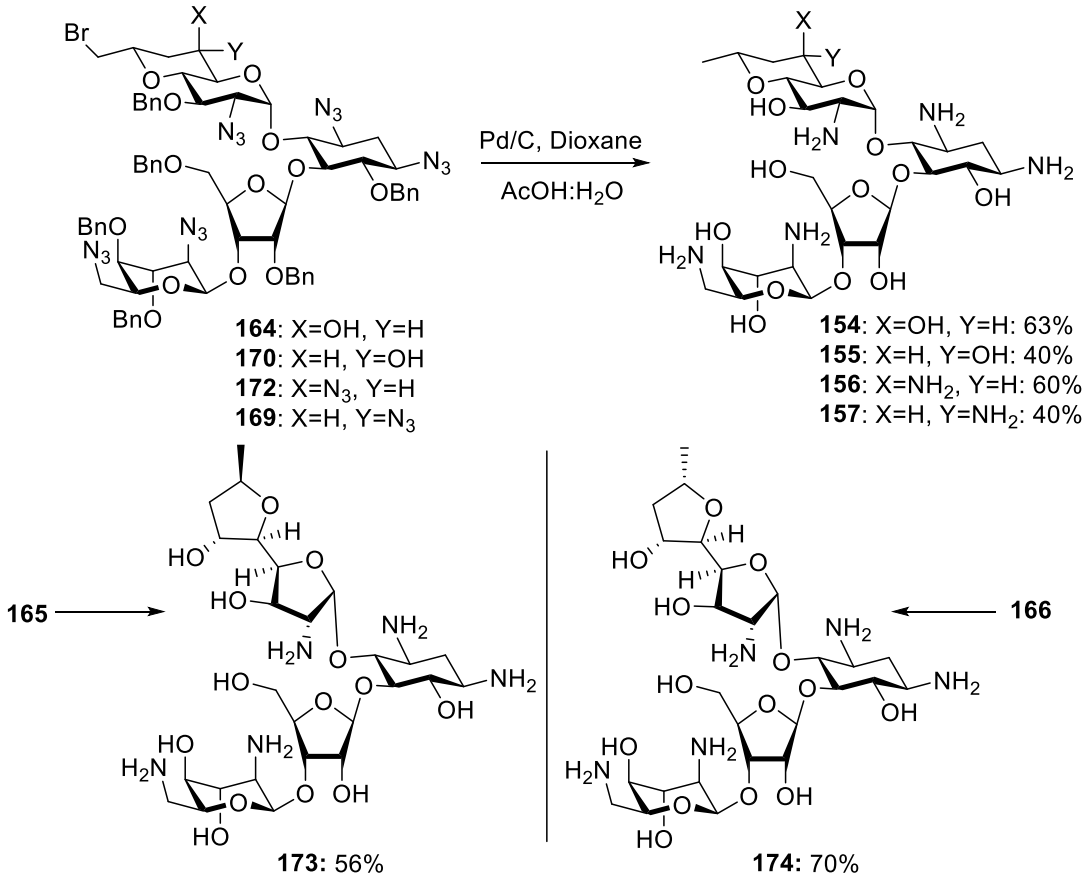
Scheme 30: **Preparation of bicyclic 6'-equatorial hydroxy, 6'-equatorial and 6'-axial azido derivatives of paromomycin**

Global deprotection was achieved in one pot by hydrogenolysis. Thus, all benzyl ethers, azido groups and the bromine atom were removed by hydrogenolysis over palladium on carbon at 40 psi in aqueous 1,4-dioxane in the presence of acetic acid. In this manner the paromomycin derivatives **154-157** with the apramycin-like scaffold for ring 1 together with the furanosyl derivatives **173**, **174** (Scheme 31) were obtained in the form of their acetate salts after purification by Sephadex resin column. These compounds were used for screening in biological assays. The relative configuration of the furanosyl derivatives **173**, **174** was assigned based on the ^{1}H, and^{13}C chemical shifts and coupling constants (Table 6), which confirmed that the both derivatives as 1,2-*cis*-glycosides.[128] In addition, the nOe spectrum of **173** showed mutual enhancement of the resonances for H-6' and H-9' on irradiation of the other proton. In contrast

the nOe spectrum of **174** showed enhancement of the resonance for H-6' on irradiation of the 8' proton.

Table 6: **1'-H NMR data of 173 and 174 derivatives**

Compound	^{1}H Chemical shift and coupling constant of 1'-H	^{13}C chemical shift of 1'-C
173	5.61 (5.14 Hz)	101.23
174	5.66 (5.14 Hz)	101.1



Scheme 31: **Global deprotection by hydrogenolysis**

3.5. Biological results

The above synthesized samples were submitted to the Böttger lab in Zurich, where they were screened for antiribosomal and antibacterial activity. The methods were identical to the ones applied in the apramycin series (Chapter 2) for the study of ribosomal susceptibility to the drug.

3.5.1. Discussion of antiribosomal activity

Table 7: **Antiribosomal activities (IC50, µg/mL) and selectivities***

Compd	**Substitution Type**	**Bacterial Activity**	**Mit13 Activity**	**A1555G Activity**	**Cyt 14 Activity**
10	Paromomycin	0.03	50.54 (2509)	5.83 (194)	10.39 (470)
18	Apramycin	0.09	67.29 (747)	27.77 (308)	58.65 (651)
14	Neomycin B	0.02	1.62 (162)	0.22 (22)	17.12 (1712)
106	6'-epiapramycin	0.74	124.21 (168)	45.08 (61)	90.01 (122)
153	4',6'-*O*-Ethylylidene	0.12	226.38 (1889)	76.97 (641)	--
154	Bicyclic 6'-axial hydroxy	0.47	193.33 (411)	213.00 (453)	169.16 (360)
155	Bicyclic 6'-equatorial hydroxy	0.02	231.85 (11593)	11.82 (591)	15.07 (753)
156	Bicyclic 6'-axial amino	0.37	2.99 (8.1)	1.66 (4.5)	10.31 (28)
157	Bicyclic 6'-equatorial amino	0.08	1.15 (14)	0.18 (2.3)	12.03 (150)

173	Furanosyl derivative-1	128	190.47	190.95	172.78
174	Furanosyl derivative-2	>128	273.31	431.01	312.40

*Selectivities are obtained by dividing the eukaryotic activity by bacterial activity.

The bicyclic paromomycin derivative **155** with the equatorial 6'-hydroxy group shows greater activity against wild-type bacterial ribosomes than the parent paromomycin **10**. In contrast the epimer **154** with the axial hydroxyl group is significantly less active. Overall it is clear that the bicyclic derivatives benefit significantly from the presence of an equatorial hydroxyl group at the 6'-position. This is because an equatorial 6'-hydroxyl group is preorganized in the *gt* conformation needed for binding to A1408 in the type I pseudo base pair (Figure 23). This preorganized hydrogen bond is sufficient to overcome any loss of affinity caused by additional hydrophobic ring and the simultaneous loss of the hydrogen bond between the 4'-hydroxyl of paromomycin and the backbone phosphate linking G1491 to A1492.

In the analogous 6'-amino series the difference in activity between the two epimers is much smaller. Furthermore the most active of the two isomers **157**, with its equatorial group, does not rise to the level of activity of the parent paromomycin. Preorganization into the *gt* conformation is therefore less beneficial for the amine than for the alcohols.

This discrepancy is explained by consideration of the ground state conformations of the ring I side chains in 6'-hydroxy and 6'-aminopyranosides and the correspondingly different energetic penalties paid on binding to the bacterial decoding A site in the *gt* conformation. Thus, based on comparison with α-D-glucosides, in aqueous solution the hydroxymethyl group of paromomycin exists as a 60:40:0 gg:gt:tg mixture of conformers; an energetic penalty is therefore paid when the gt conformer is imposed in the type I pseudo-base pair interaction. This

energetic penalty is removed in the bicyclic derivative **155** and binding is correspondingly enhanced. On the other hand the protonated aminomethyl side chain of neomycin is expected to exist as a 10:90:0 mixture of the *gg*, *gt* and *tg* conformers (Figure 20A) based on comparison with 6-amino-α-D-glucosides.[119] The aminomethyl group of neomycin is therefore already preorganized for formation of the type I pseudo-base pair and there is no advantage to be gained from enforcing it in a bicyclic derivative.

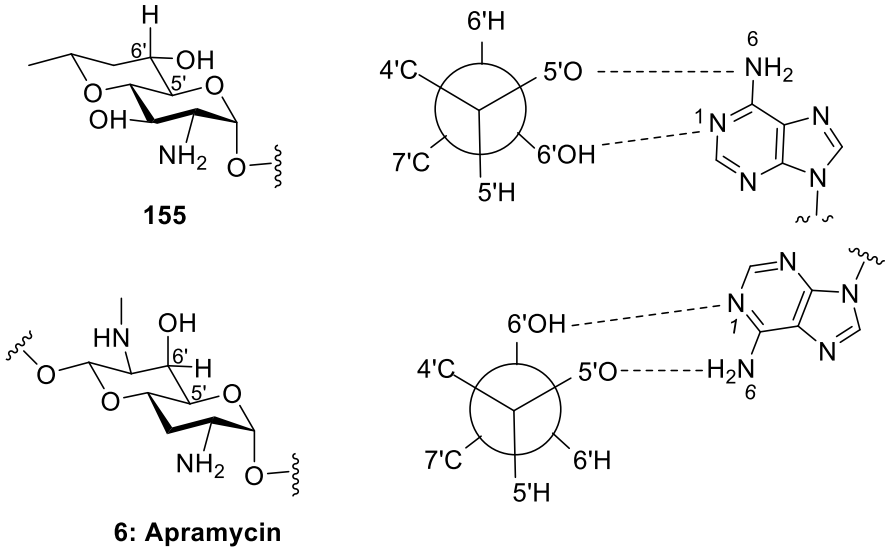


Figure 23: **Binding pattern of bicyclic bicyclic 6'-equatorial paromomycin 155 with A1408 base and apramycin with A1408**

Furthermore, the 6'-equatorial hydroxyl paromomycin derivative **155** exhibits remarkable selectivity against the eukaryotic mitochondrial ribosome and better selectivity over the A1555G mitochondrial mutant ribosome which is highly susceptible to AGA induced ototoxicity. Such dissimilarities in affinity for the prokaryotic and mitochondrial ribosomes are consequence of the differing interactions of the β-face of ring I with the nucleotide bases at the bottom of the target decoding A site. Therefore, the base pair G1491=C1409 at the bottom of the bacterial A site, in particular, the affinity between G1491 and the β-face of the AGA ring I through the CH-π interactions compensates for any kind of loss due to the introduction of the hydrophobic bicyclic

moiety. Conversely, the mitochondrial ribosome built with two consecutive non Watson-Crick pairs (C1491•C1409 and A1490•C1410) cannot compensate as much for the introduction of a hydrophobic bicyclic ring I. On the other hand, the both isomers of the 6'-amino analogues **156, 157** strongly inhibit the mitochondrial ribosome and its A1555G mutant, which is similar to the parent neomycin.

Further, the 6'-equatorial hydroxyl paromomycin derivative **155** displays better selectivity than the 6'-axial analogue **154** over the eukaryotic cytoplasmic ribosomal A site whereas, the analogous 6'-amino compounds exhibit lower affinity towards the cytoplasmic decoding A site. This is due to the mutation A1408G that does not permit the pseudo-base pair with the 6'-amine of the ring I AGA, due to repulsions between protonated amines.[129]

Finally, both the unusual five membered furanosyl derivatives (**173**, **174**) display total loss of activity against all ribosomes, which is probably due to the fact that the two five membered rings cannot be accommodated in the drug binding pockets.

Overall, based on the antiribosomal data, it is confirmed that the rigid bicyclic derivatives of paromomycin with equatorial 6'-hydroxyl **155** and 6'-amino compound **157** bind tighter to the ribosomal decoding A site than their axial analogues **154** and **156**. These observations are in contrast with the unusual bicyclic AGA apramycin, with the axial 6'-hydroxy group, which binds more tightly than the equatorial isomer (Chapter 2). The origin of the differences between the bicyclic paromomycin derivatives described in this chapter for which an equatorial 6'-hydroxyl group is clearly preferred and the apramycin series when the axial isomer await further investigation.

3.5.2. Discussion of antibacterial activity

All paromomycin derivatives prepared, together with the parent paromomycin **1** and other comparators apramycin, and neomycin, were screened for antibacterial activity against clinical isolates of methicillin-resistant strains of the Gram-positive bacterium *Staphylococcus aureus* and clinical isolates of the Gram-negative bacterium *Escherichia coli*. Consistent with the promising results in the cell-free translation assays, the bicyclic 6'-epiparomomycin derivative **155** displayed greatest activity against all clinical strains of MRSA and/or *E coli*. Replacement of the 6'-hydroxyl group with an amine **156**, leads to significant activity against all strains of *S aureus* and/or *E coli*, with even better values observed than parent compounds, while the axial isomer **157** is much less active. Further, the unusual 5-membered compounds displayed no activity against all tested strains (Table 8). Overall, with the exception of **157** all bicyclic derivatives **(154, 155, 156)** display good activity against two clinical strains of MRSA (AG039, AG042), that are resistant to the parent antibiotic. This activity enhancement is a result of interfering with the resistance mechanism of two MRSA strains by drug modification of either ANT (4') or APH (3') AMEs.

Table 8: **Antibacterial Activities (MIC, µg/mL)**

Compd	***MRSA***					***E coli***		
	AG038	AG039	AG042	AG044		AG001	AG055	AG003
Paromomycin	4	>256	>256	4-8		16-32	8	8-16
Neomycin	0.5-1	128	128	0.5-1		8-16	4	4
Apramycin	8	8	8	16		16	8	8-16
106	32-64	64	64	32-64		32	32	32
153	32	64	32	32		>128	-	64-128
155	8-16	8	8	4		8	8	8
154	32	32-64	16-32	32		≥128	64-128	64-128
156	4	4	4	2		2	2	2
157	>128	>128	128	128		>128	>128	>128
173	>128	>128	>128	>128		>128	>128	>128
174	>128	>128	>128	>128		>128	>128	>128

3.6. Conclusions

A new class of paromomycin antibiotics was designed based on the existing active molecule library. This new design focused on the modification of ring I at the 4' and 6'-positions. It consists of an apramycin-like bicyclic scaffold and a key hydroxy group or amine at 6'-position to bind to the RNA bases. All these new targets of paramomycin antibiotics were synthesized and screened for antiribosomal activity in cell-free translation assays with a series of wild-type and human mitochondrial and cytosolic ribosome models, as well as for antibacterial activity against clinical isolates of *E-coli* and methicillin-resistant *Staphylococcus aureus*. A bicyclic

derivative with a 6'-equatorial hydroxyl displays better activity against the bacterial wild-type ribosome than the original paromomycin. The information from the trend of activity changes between locked systems and free side chain models contributes to the understanding of binding pattern of AGAs with the bacterial A site and will be helpful for the future drug design.

CHAPTER 4. INFLUENCE OF THE ISOTHIOCYANATO MOIETY ON THE STEREOSELECTIVITY OF SIALIC ACID GLYCOSIDES FORMATION AND ITS USE IN SUBSEQUENT DIVERSIFICATION

4.1. Introduction to sialic acids

Sialic acids are higher carbon sugars found at the outer most position of the glycoprotiens and glycoconjugates where they play an important role in various biological process.[130] They are a group of nonulosonic acids featuring an anomeric carboxylic acid and a deoxygenated C-3 methylene group. The sialic acids are linked to glycan chains via specific glycosidic linkages.[131] Naturally occurring sialic acids exist in different forms varying in the substitution of the pyranose skeleton, by modifications of the hydroxyl groups, and through the anomeric sialyl linkages affixing them to different types of glycans. Mostly, three common forms of sialic acids are available; *N*-acetyl neuraminic acid **175**, *N*-glycolylneuraminic acid **176** and keto-deoxy-nonulosonic acid **177**. Neu5Ac **175** is a nine carbon deoxy sugar with an acetamido substituent at the C-5 position; it is the most abundant sialic acid and is extensively distributed in nature.[130]

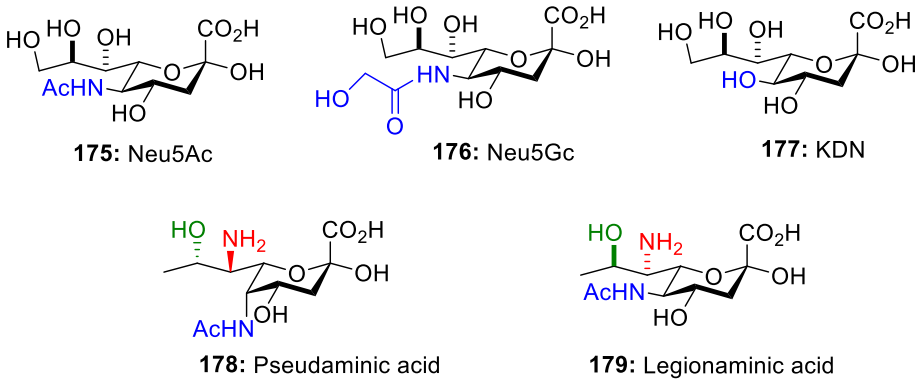


Figure 24: **Naturally existing sialic acids**

In addition, post translational alterations of the sialic acids, such as acetylation and phosphorylation at C-9 and methylation and sulfation at C-8, expand the diversity of these molecules to the extent that more than 50 sialic acid derivatives have been found in nature.[132]

Pseudaminic acid **178** and legionaminic acid **179** are two 9-deoxy-7-amino derivatives of the sialic acid scaffold found in bacteria, generally at the non-terminal positions of bacterial glycans (Figure 24).[133]

4.2. Linkage diversity and biological importance of sialic acids

Mammalian sialic acids occur in limited linkage modes and their linkage diversity is well documented.[132] The naturally existing equatorial sialic acid glycosides are classified as the α-anomers, while the artificial axial glycosides are classified as the β-anomers. Sialic acids are most commonly α-linked to the 3- and 6- positions of galactopyranose or the 6-position of galactosamine. Another important linkage form of the sialic acids is the homopolymeric form, which observed in bacteria when multiple residues are joined to each other in the α(2→8) or α(2→9) fashions. The anomeric carboxylic group of the sialic acids confers negative charge on molecule into which they are incorporated under physiological conditions, but is also found lactonized with hydroxyl groups on the adjacent residues as in compound **183** (Figure 25).[134] The CMP-sialic acid sugar nucleotide **184** is used as a glycosyl donor by the sialyltransferases in the biosynthetic pathway of the sialyl glycans and is the only sialoside with a β-linkage in mammals.

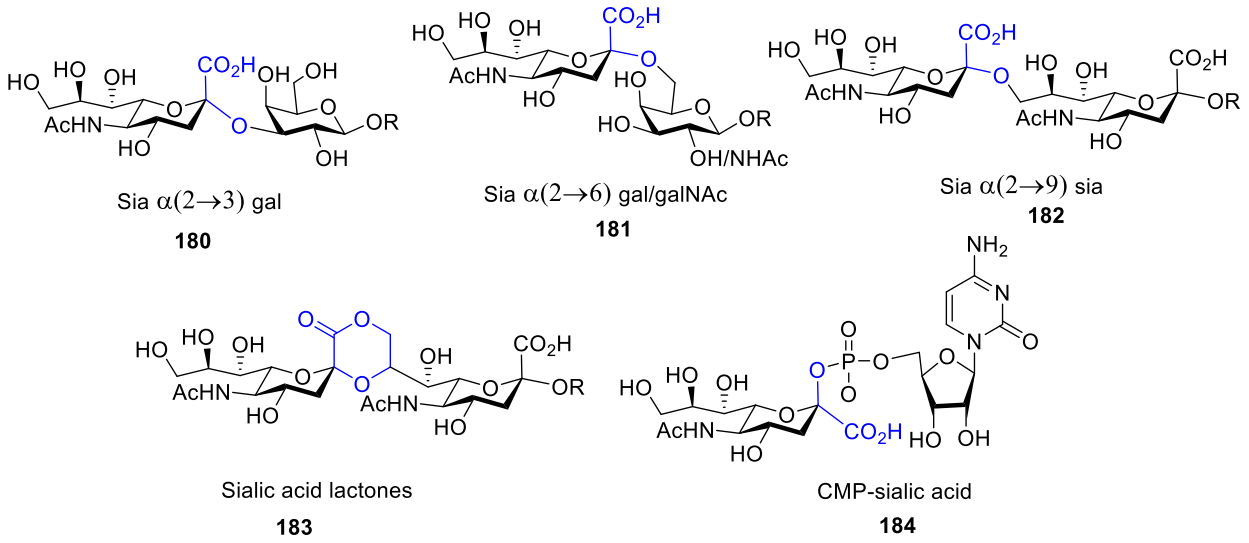


Figure 25: **Diversity in the naturally existing sialic acid linkages**

Sialic acids perform a multitude of biological functions. In particular, Neu5Ac and KDN are two sialic acids found in the human biological regime.[135] These sialic acids execute functions ranging from normal physiochemical effects on the cellular environment to specific phenomena relating to molecular and cellular recognition, as a function of their charge, size and hydrophilic nature.[135] Sialic acid functions can be divided into two groups; firstly, they can act as a biological recognition sites or receptors as in their binding of viral influenza causing lectins.[136] Secondly, they can also act as biological masks by shielding the recognition sites such as penultimate sugars of glycan.[137] Sialic acids can prevent erythrocytes from degradation by masking the subterminal galactose residues.

The sialidase enzymes remove the terminal sialic acid from cell surface glycans. This is a key step in the replication cycle of influenza viruses and which gives importance to the sialidase inhibitors as antiviral drugs. They are associated with different pathological processes like cholera, influenza and Salla disease.[138] Increased understanding of the bacterial and viral

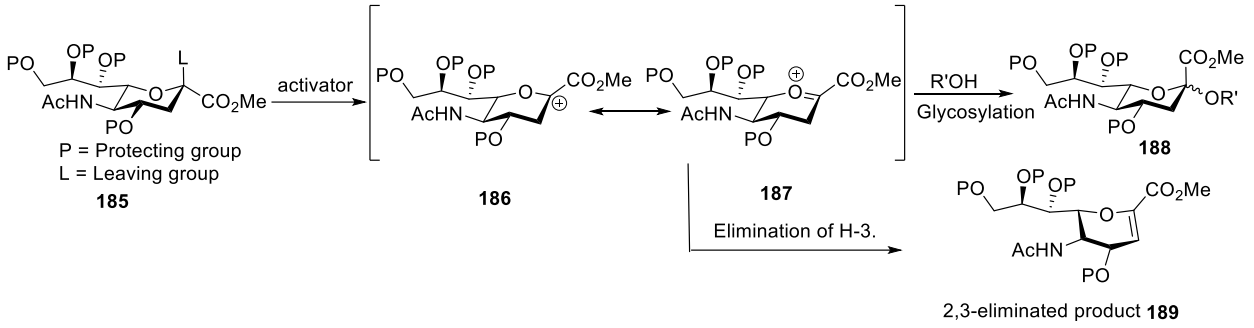
neuraminidases has led to the rational design and synthesis[139] of sialic acid based drugs like Zanamavir and Tamiflu.[140]

4.3. Synthesis of sialic acid glycoconjugates

The sialome, which is a subclass of the glycome, is defined as the complete study of the sialic acids, their linkages and modes of action. The current understanding of the sialome, in particular of the vast number of roles played by sialic acids in vertebrates, leaves many challenges in the development of methods to obtain well characterized sialic acid containing glycoconjugates.[141] The study of the biological functions of the sialylglycans requires structurally defined homogeneous molecules, but the isolation of pure forms of glycans from natural sources is very difficult owing to the heterogeneity and diversity of these molecules. These circumstances highlight the importance of the development of efficient enzymatic or chemical methodologies for the synthesis homogenous glycans.[134] Although chemoenzymatic methods offer high substrate promiscuity in the synthesis of sialyl glycoconjugates, the development of synthetic chemical methods enjoys a lead role due to the more significant quantities it can provide as well as the ability to access non-natural linkages.

The chemical synthesis of the naturally occurring α-linked sialosides involves many challenges. These challenges arise primarily from the presence of the electron withdrawing carboxylic acid functionality at the anomeric position and the lack of functionality at the 3-position, which together lead to a number of complications on activation. These complications include the formation of the 2,3-eliminated product **189** following oxocarbenium ion **187** formation (Scheme 32). As with all equatorial glycosides the anomeric effect has to be circumvented in the formation of the α-sialosides. This is complicated by the absence of a functional group at the 3-position precludes the possibility of any kind of stereodirecting

participation from that position unless significant modifications are made to the donor. As a result of the combination of these factors, glycosylation reactions of sialic acids are often low yielding and poorly selective.

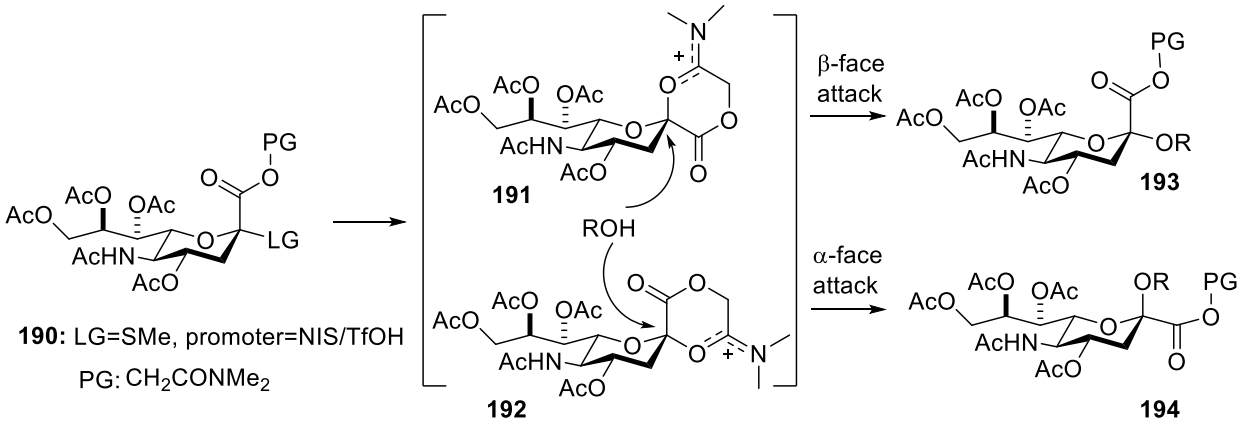


Scheme 32: **General glycosylation or sialylation reaction**

The chemical synthesis of the α-sialic acid linkages has long been of considerable synthetic interest, which is reflected in the numerous strategies that have been developed toward the establishment of high-yielding and highly α-selective chemical sialidation reactions. The various approaches that have been employed to overcome this problem can be categorized into, i) auxiliary group assisted sialylation, ii) modification of the natural N-5 acetyl group by installation of electron withdrawing groups, and iii) use of cyclic protecting groups.

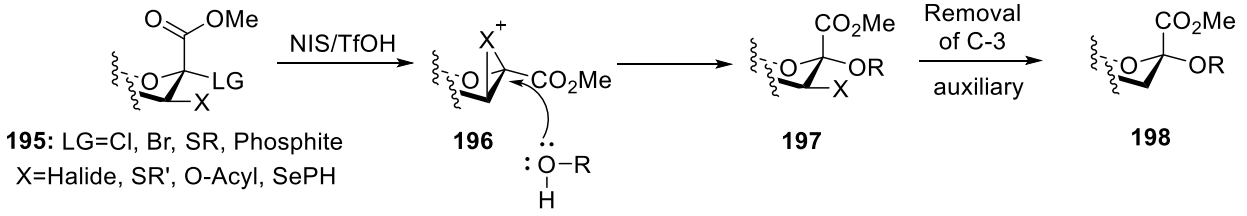
4.3.1. Auxiliary group assisted sialylation

Neighboring group assisted glycosylation is a popular technique and widely used to control stereoselectivity.[142] Modification of sialyl donors by incorporation of auxiliary groups at the C-1 and C-3 positions to improve the glycosylation reaction profile is an important strategy in the field. C-1 auxiliaries have been explored by the Gin[143] (Scheme 33) and Takahashi[144] groups who used *N*,*N*-dimethylglycolamide and 2-thioethyl ester participating groups, respectively.



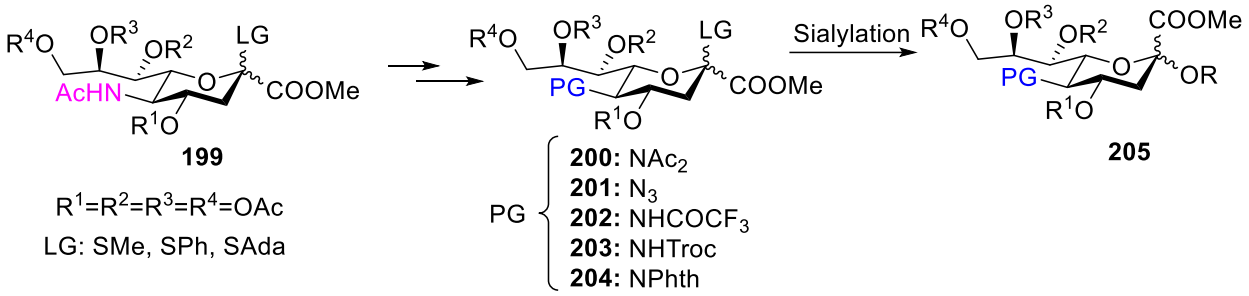
Scheme 33: **C-1 auxiliary glycosylation (Gin's approach)**

Gin's sialylation using an *N,N*-dimethylglycolamide auxiliary **190** (Scheme 33) was somewhat selective for primary alcohol acceptors, but it showed only modest α-selectivity with hindered secondary alcohol acceptors. [143] The related Takahashi approach, with participation by a thioether gave only very modest selectivity.[144] The use of auxiliaries at the 3-position is complicated due to need to installation and eventually remove the auxiliary on a methylene group. Examples of the class include halides, acetoxy groups, thioethers and phenyl selenides (**195**) with some affording α-sialosides successfully albeit in a leaving group dependant manner (Scheme 34).[145] Although these methods are useful in that they give modest α-selectivity, they are considered less attractive due to the further steps required to remove the auxiliary groups.



Scheme 34: **C-3 auxiliary supported glycosylation**

4.3.2. Replacing the *N*-5 acetamide by electron withdrawing groups



Scheme 35: **General scheme for the modification of N-5 substituent followed by the sialylation**

The chemical modification of C-5 position by the introduction of various protecting groups results in a change of reactivity and stereoselectivity of the glycosylation reaction.[146] In particular, the incorporation of electron withdrawing groups at C-5 position has great influence on the stereoselectivity of sialylation. The introduction of a further acetyl group on Neu5Ac, as in the acetimide **200**, is achieved by the simple acetylation of fully deprotected Neu5Ac with concomitant *O*-acetylation. The higher reactivity of the **200** in comparison with the mono-*N*-acetylated donor **199** was reported for the synthesis of α-(2→3) linked disaccharides and α-(2→8) linked dimers.[147,148] Also, Crich *et al.* shown that the challenging α-sialylation with 5-*N*-acetylacetamido derivative **200** can be efficiently performed by using the diphenyl sulfoxide/trifluoromethanesulfonic anhydride activation system in the absence of acetonitrile. With this glycosylation method, the Neu5Ac α(2→6) Gal glycosidic linkages can be installed with excellent yield and selectivity.[149] 5-Azido derivatives of neuraminic acid **201** have been obtained by the biosynthetic method[150] and by chemical methods.[151] Higher stereoselectivities have also been reported for the synthesis of α(2→6) and α(2→9) dimers with the 5-azido donors.[152-155] Higher stereoselectivity with the 5-trifluoroacetamido donor **202** has also been reported in the synthesis of α(2→8) and α(2→9) linked dimers and oligosaccharides.[154,156-158] Similarly, the replacement of the acetamide group with other electron withdrawing groups as in

the 5-*N*-Troc **203**,[159-161] 5-*N*-phthalimido **204**,[162-165] *N*-glycolyl,[166] *N*-*t*-butyloxycarbonyl (Boc),[167] *N*-benzyloxycarbonyl (Cbz),[168] *N*-t-butyloxycarbonylacetamido (NAcBoc),[169,170] and *N*-Fmoc, *N*-Alloc, and trichloroacetyl[160,171] groups has been shown to increase α-selectivity in the synthesis of oligosaccharides (Scheme 35). Overall the placement of an electron withdrawing group at the C-5 position has a significant impact on the α-selectivity with primary alcohol acceptors. Nevertheless many of these methods are associated with limitations to the use of unhindered primary alcohol acceptors.

4.3.3. Use of cyclic protecting groups to attain α-selectivity

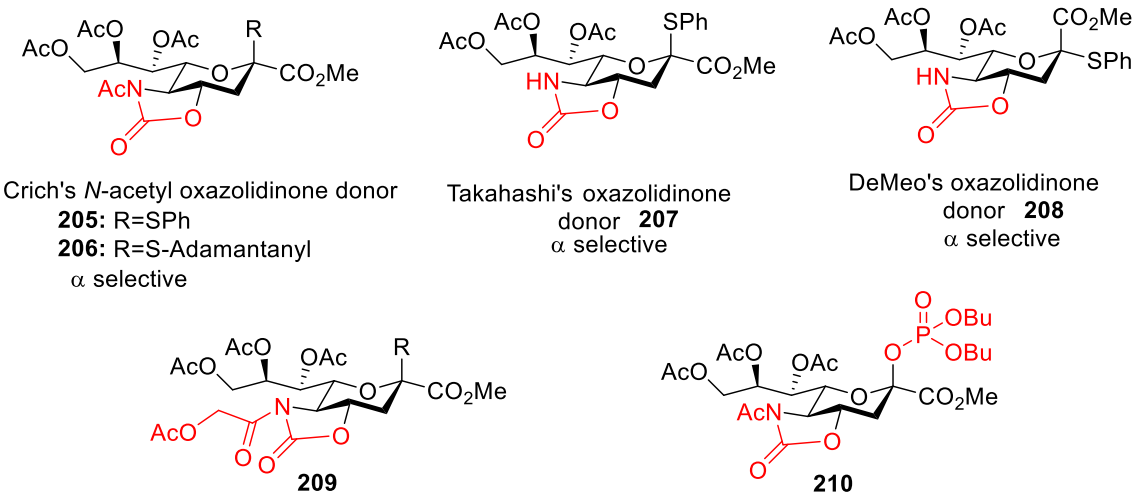
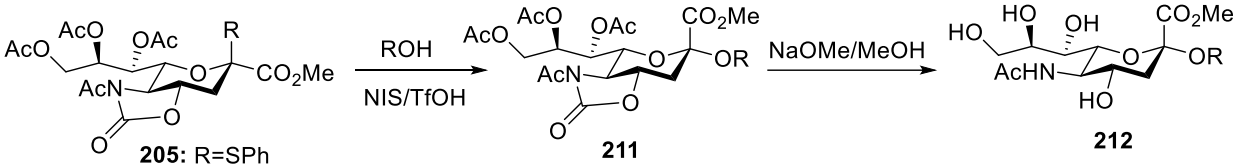


***Figure 26:* 4*O*, 5*N* cyclic protected sialyl donors**

A significant breakthrough in the area of α-selective sialylation arose from the introduction of cyclic protecting groups spanning 4*O* and 5*N* of sialyl donors. Crich *et al.* developed a α-sialylation donor which features a *trans*-fused *N*-acetyl 5*N*,4*O*-oxazolidinone protected phenylthio sialoside **205** or the thioadamantyl sialoside **206**. These donors offer excellent stereochemical control of glycosylation as well as excellent yields under the NIS/TfOH in situ activation conditions.[172,173] These donors can be directly used for glycosylation, without a

need of any auxiliary functionality to control the stereo selectivity. The advantage of the extra *N*-acetyl group in the Crich method arises from the mild conditions used for the cleavage of the *N*-acetyl 5*N*,4*O*-oxazolidinone with direct regeneration of the native C-5 acetamide **212** (Scheme 36). This is to be contrasted with the otherwise excellent donors from the Takahashi[174] and De Meo[175] laboratories based on the simple 5*N*, 4*O*-oxazolidinones **207** and **208,** which require harsh conditions for cleavage of the 4,5-*O*,*N*-oxazolidinone ring.

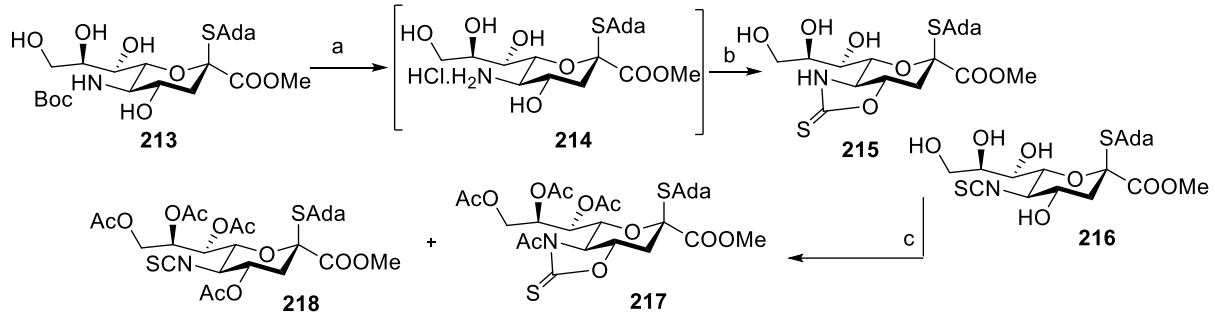


205: R=SPh
206: R=S-Adamantanyl

211 **212**

Scheme 36: **Glycosylation followed by Zemplen deacetylation of Crich's *N*-acetyl oxazolidinone sialosides**

The Crich group also extended the outstanding stereoselectivity of the *N*-acteyl oxazolidinone donor **206** to the analogous *N*-glycolyl oxazolidinone donor **209**, and reported a one-pot glycosylation method to construct oligosaccharides containing Neu5Gc at the terminal position.[176] Further, the same group reported the synthesis of C- and S- α-sialosides[177,178] using Wong's *N*-acteyl oxazolidinone protected sialyl phosphate **210**[179] using the milder TMSOTf conditions for activation thereby enabling the synthesis of unnatural sialosides with excellent α-selectivity.



Scheme 37: **Synthesis of 5*N*,4*O*-Oxazolidinthione and isothiocyanate derivatives**

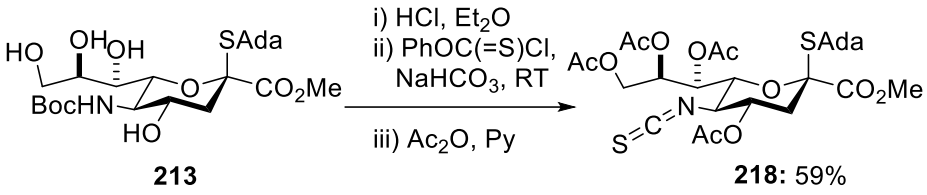
Seeking to extend the concept of cyclic protecting groups to the more strongly electron withdrawing *N*-acetyl oxazolidinthione protected system **217** Crich and coworkers found that higher temperatures were required for activation, resulting in lower selectivities overall.[180] The synthesis of *N*-acetyl oxazolidinthione-protected sialyl thioglycoside **217** was accomplished from the known neuraminic acid intermediate **213** by treatment with HCl in ether to give **214**, followed by treatment with phenyl thionochloroformate and sodium hydrogen carbonate in aqueous acetonitrile yielding intermediates **215** and **216**. Further acetylation with sodium hydride and acetyl chloride then furnished donor **217.** This synthesis provided a byproduct, the isothiocyanate **218**, which arises from the incomplete cyclization of the intermediate phenyl thionocarbamate (Scheme 37).

This chapter describes the exploration of the isothiocyanate **218** as a sialyl donor, and subsequent work taking advantage of the diverse reactivity of the isothiocyanate for the preparation of sialosides diversely functionalized at the 5-position.

4.4. Results and Discussion

4.4.1. Synthesis of an isothiocyanate protected donor

As described above, the peracetyl adamantanyl thiosialoside **218** protected by an isothiocyanate group at the *N*-5 position was initially isolated as a by-product in the synthesis of the *N*-acetyl-4-*O*,5-*N*-oxazolidinthione protected sialyl donor **217**.[180] In an improved synthesis, the β-*S*-adamantanyl thiosialoside **213** was treated with HCl in diethyl ether, followed by phenyl chlorothionoformate and aqueous sodium bicarbonate at room temperature, and finally acetic anhydride in pyridine to give the target **218** in 59 % yield (Scheme 38). Isothiocyanate **218** is a stable white crystalline solid, which can be readily handled and stored.



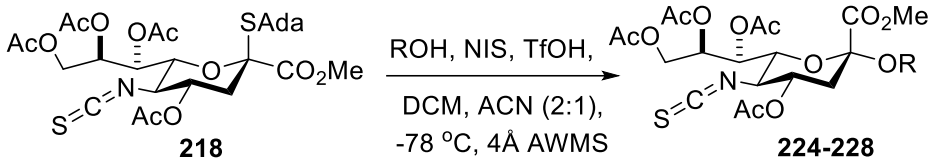
213 **218:** 59%

Scheme 38: **Synthesis of the isothiocyanato donor 218**

4.4.2. Sialylation using isothiocyanato donor 218

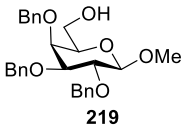
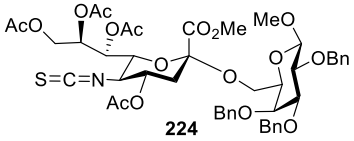
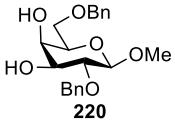
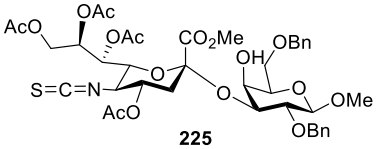
To study the influence of the isothiocyanate group on the stereoselectivity of sialylation, a series of reactions were performed using isothiocyanate donor **218** and a range of acceptors. Activation of **218** in the presence of 1.2 equiv. of various acceptors using NIS/TfOH activation system afforded the corresponding glycosides, exclusively as the α-anomers (Scheme 39, Table 9). The glycosylation of monosaccharide acceptors such as the galactopyranosyl 6-ol **219**, the galactopyranosyl 3,4-diol **220**, and the galactopyranosyl 3-ol **221** with **218** using standard glycosylation conditions gave the disaccharides **224**, **225** and **226,** respectively, in a highly stereoselective manner and excellent yield. In particular, the glycosylation of the 4-*O*-protected galactopyranosyl 3-OH acceptor (Entry 3, Table 9) is noteworthy as it gave the coupled product

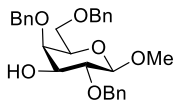
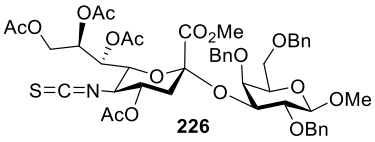
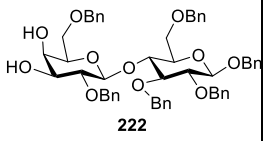
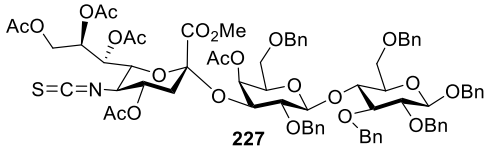
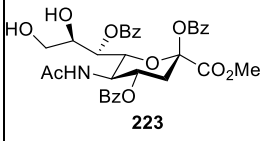
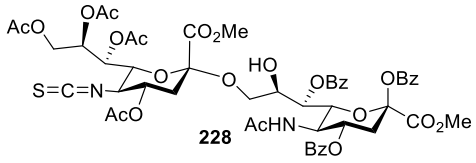
as a single anomer **226**. This is be contrasted with the typically poorly selective coupling of **221** to other sialyl donors, including the *N*-acetyl oxazolidinones**.** The isothiocyanate donor **218** was also coupled with the di- and tri- saccharyl acceptors **222** and **223** and gave the α-anomers of the products in 55% and 58% yields, respectively (Entry 4 and 5, Table 9). Overall, these experiments showed that the isothiocyanato donor **218** is highly beneficial in imparting α-selectivity. The anomeric configuration of the resulting glycosides was assigned on the basis of the heteronuclear $^{3}J_{C1,H3ax}$ coupling constant method as discussed below.[181-185]



Scheme 39: **Glycosylation with isothiocyanate 218**

Table 9: **Glycosylation with per-acetylated isothiocyanate donor**

Entry	**Acceptor**	**Product**	**Yield & Selectivity**	**Coupling constant ($^{3}J_{C1-H3ax}$)**
1	 **219**	 **224**	80% (α-only)	6.7 Hz
2	 **220**	 **225**	79% (α-only)	7.5 Hz

3	 **221**	 **226**	87% (α-only)	7.0 Hz
4	 **222**	 **227**	55% (α-only)	6.5 Hz
5	 **223**	 **228**	58% (α-only)	6.5 Hz

4.4.3. Assignment of anomeric configuration for coupled products

The commonly used NMR methods for the assignment of anomeric configuration of glycopyranosides, such as the evaluation of $^{3}J_{H1\text{-}H2}$ and $^{1}J_{C1\text{-}H1}$ NMR coupling constants,[186] are not be suitable for the sialic acid glycosides due to the absence of an anomeric proton. For this reason numerous alternative methods have been reported in the literature to determine the anomeric configuration of sialosides based on i) the chemical shift of the *H*-3_{eq},[187] and *H*-4[188,189] resonances, ii) the Δδ value of the resonances for *H*9a-*H*9b,[190] iii) the δ values of *H*-7 and *H*-8,[189,190] and the measurement of $^{3}J_{C\text{-}1,\ H3ax}$ heteronuclear coupling constants.[181-185] Among these methods, the measurement of $^{3}J_{C\text{-}1,\ H3ax}$ coupling constant is the most reliable as it is based on the correlation of coupling constants with torsional angles and not on the interpretation of chemical shift differences which are affected by many factors. Thus, the method differentiates between α

and β-sialosides on the basis of the respective numerical values of 5-7 Hz and 0-2 Hz of the $^{3}J_{C\text{-}1,\ H3ax}$ coupling constant. This difference in the coupling constants can be explained based on the Karplus relationship for $^{3}J_{C\text{-}1,\ H3ax}$ of the sialoside anomers. In the $^{2}C_{5}$ chair form, the dihedral angles of C1-C2-C3-H3ax of the α and β-anomers are 180º and 60º, respectively (Figure 27).[182]

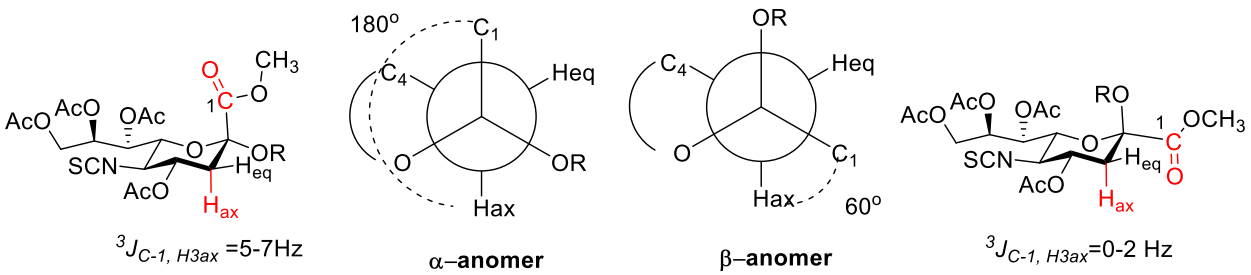


Figure 27: **Dihedral angles of α and β-anomers of sialosides**

The practical implementation of the method is illustrated for **228** in Figure 28 where three NMR experiments were used to measure the $^{3}J_{C\text{-}1,\ H3ax}$ coupling constants. First, the standard broad band proton decoupled carbon spectrum shows 10 carbonyl signals in the down-field region (164–172 ppm). Second, a spectrum recorded with the broadband decoupler turned off gives the complete proton coupling profile for all of the carbonyl carbons. Finally, a ^{13}C NMR spectrum obtained with selective decoupling of the C-1 methyl ester protons which reveals the residual doublet nature of the C-1 signal at δ 167.5 owing to coupling to the axial hydrogen at C3. The observed coupling constant of 6.5 Hz for this doublet leads indicates C-1 and H3ax to have an antiperiplanar relationship and consequently the glycoside to have the α-configuration.

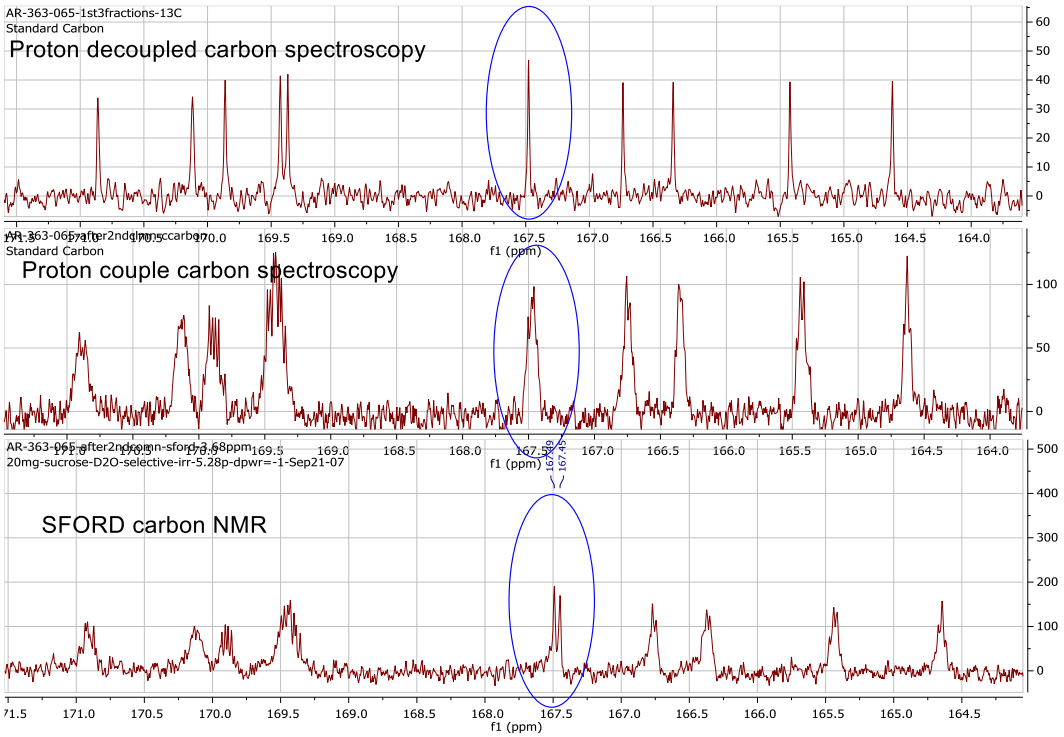
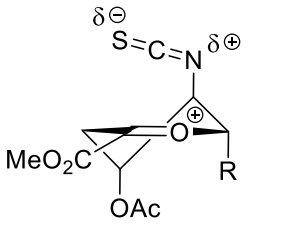


Figure 28: **Sialoside 228 stereochemical assignment using the $^{3}J_{C\text{-}H}$ coupling constant method**

4.4.4. Selectivity

In order to explain the selectivity of the isothiocyanate **218**, two possibilities can be considered. First, it is possible that the isothiocyanate simply acts as a strongly electron-withdrawing group and promotes S_N2-like glycosylation as has been proposed for the oxazolidinone system on the basis of mass spectrometric fragmentation studies.[191] In this respect it is noteworthy that the isothiocyanate group is considerably more polar than the azido and isocyanate groups (dipole moments of $C_6H_5N_3$, $C_6H_5N{=}C{=}O$, and $PhN{=}C{=}S$ in Debye units, respectively: 1.82, 2.43, 2.69).[192,193] Alternatively, an explanation can be advanced based on through space stabilization of glycosyl oxocarbenium ions in an inverted conformation.[194,195] In this second possibility the transient intermediate sialyl oxocarbenium ion **229** is considered to adopts the $^{5}H_4$ conformation preferentially to take advantage from stabilization by the

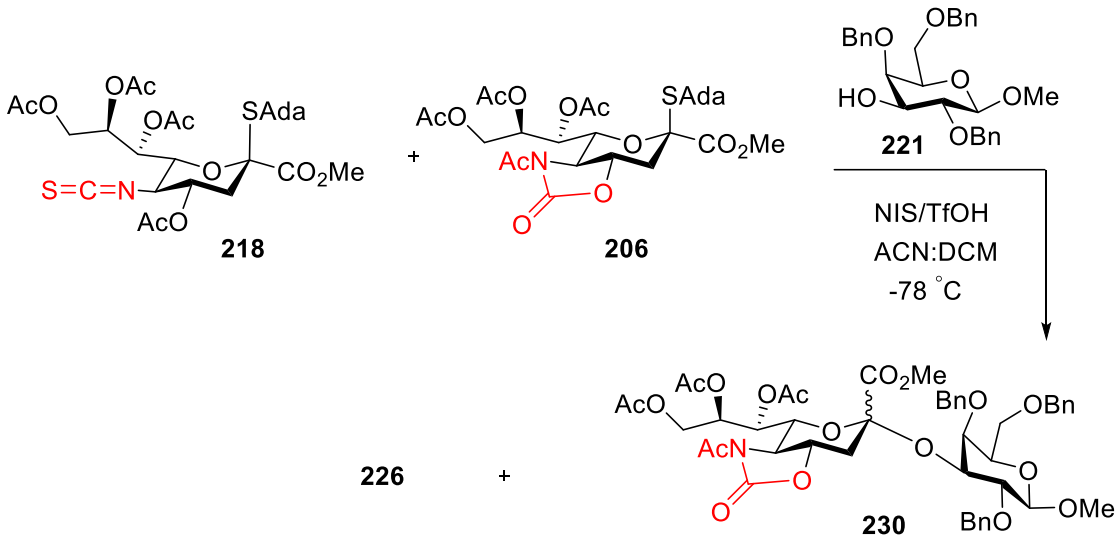
pseudoaxial 4-*O*-acetate and the C-5 isothiocyanate groups. In such a conformation the isothiocyanate would provide significant steric shielding to the β-face of the oxocarbenium, leading to enhanced α-selectivity (Figure 29).



229

Figure 29: **Structure of the Oxocarbenium ion**

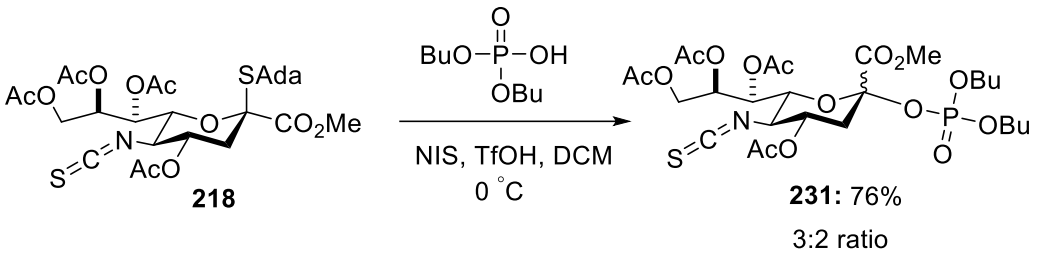
A competition experiment was designed to probe the relative reactivity of the isothiocyanate **218** and the *N*-acetyloxazolidinone **206** and thus indirectly the relative electron withdrawing effects of the protecting groups in the two systems. Accordingly, an equimolar mixture of **218**, **206** and acceptor **221** were activated with NIS/TfOH, -78 ºC followed by the standard work up leading to the isolation of the coupled products **226** (α-only) and **230** (α/β=4:1 mixture) in 3 and 51% yields, respectively. Clearly the reactivity of the isothiocyanate donor **218** is lower than that of the *N*-acetyloxazolidinone **206**, which is consistent with the highly electron-withdrawing nature of the isothiocyanate moiety (Scheme 40).



Scheme 40: **Competition experiment to estimate the relative reactivity of donors 218 and 206**

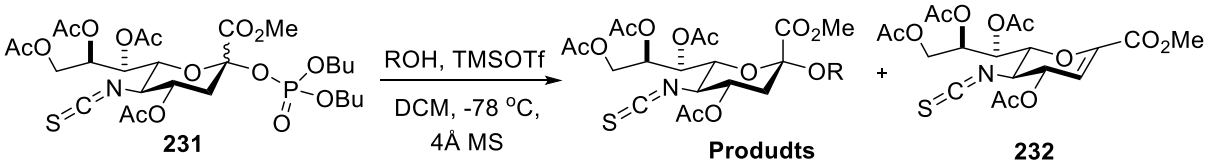
4.4.5. Synthesis and fragmentation studies of sialyl phosphates

The study of different leaving groups at the anomeric position plays a major role in the development of sialic acid donors for efficient α-sialylation methods. Apart from the thioglycosides, sialyl phosphates have been demonstrated to be an important class in the glycosylation reaction particularly used in conjunction with the oxazolidinone–type protecting groups.[179] Sialylation with sialyl phosphate donors has several advantages including high reactivity but more especially the mild reaction conditions. Indeed, the mild conditions were critical in the Crich group's demonstration of the efficient synthesis of C α-sialosides using sensitive allylstannanes and silyl enolethers as nucleophiles.[177] In view of this, the conversion of the isothiocyanate protected sialyl donor **218** to the corresponding sialyl dibutyl phosphate was investigated and the use of this novel donor in sialylation reactions and as a mass spectrometric probe were explored.



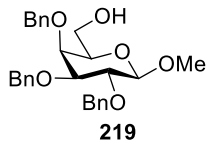
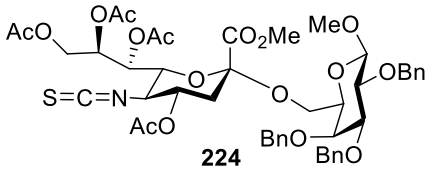
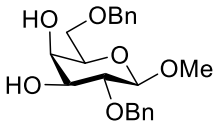
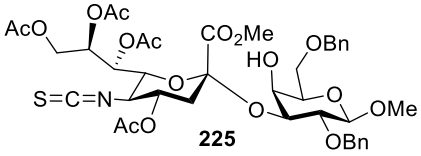
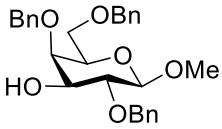
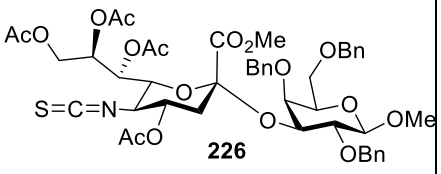
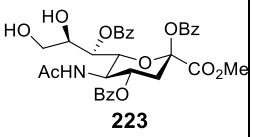
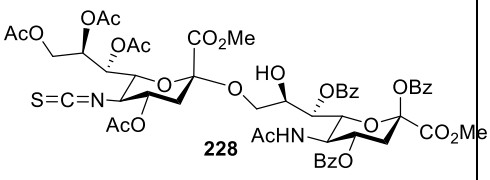
Scheme 41: **Formation of sialyl phosphates 231**

Thus, thioglycoside **218** was treated with dibutyl phosphoric acid in the presence of the NIS/TfOH activating system in DCM at 0 ºC, resulting in the isolation of the desired isothiocyanate containing sialyl phosphate **231** in 76 % yield (Scheme 41) as a 3:2 α/β mixture. Subsequently, a series of sialylations were performed using the new donor and a range of acceptors. Glycosylation reactions were conducted with **231** in the presence of 1.2 equiv. of various acceptors using TMSOTf as the activation system at -78 ºC The corresponding glycosides were obtained exclusively as the α-anomers in moderate yields (Scheme 42, Table 10). Overall, these experiments showed that the stereoselectivity already evident with the thiosialoside **218** is also operative with the sialyl phosphate **231**. Yields, however, were generally lower with the phosphate donor (Table 10) than with the thiosialoside (Table 9) due to the competing formation of the 2,3-glycal by-product **232** as judged by mass spectrometric analysis of the crude reaction mixtures.



Scheme 42: **Glycosylation with sialyl phosphate 231**

Table 10: **Glycosylation with sialyl phosphate donor**

Entry	Acceptor	Product	Yield & Selectivity
1	 **219**	 **224**	52% (α-only)
2	 **220**	 **225**	43% (α-only)
3	 **221**	 **226**	55% (α-only)
4	 **223**	 **228**	55% (α-only)

4.4.6. Mass spectral fragmental studies of sialyl phosphates

The isothiocyanate containing sialyl phosphate **231** was subjected to the in-source fragmentation experiment in order to determine the influence of the isothiocyanate on the formation of the oxocarbenium ion. [196] The technique used, is a cone-voltage induced fragmentation performed on an ESI mass spectrometer and derives from the work of Denekamp

and Sandlers on the use of threshold fragmentation energies to probe the influence of protecting groups on the stability of glycosyl oxocarbenium ions.[197,198] The Crich group previously utilized this strategy in the study of the influence of other protecting groups on the formation of sialyl oxocarbenium ions.[196] The ESI fragmentation of the phosphate probably occurs by the expulsion of dibutyl phosphate to give the corresponding oxocarbenium ion**,** which then undergoes deprotonation to give the observed 2,3-glycal fragment ion. Experimentally, various combinations of differentially protected sialyl phosphates[196] were injected in to the ESI spectrometer and the cone voltage gradually increased until fragmentation began, with the minimum detection level set to 2% of the TIC. The results reveal that compounds carrying cyclic protecting groups (**210, 235**) required more energy to attain the corresponding oxocarbenium ions when compared to those with acyclic protecting groups (**233, 231** and **235**). However, it was not possible to distinguish between the threshold energies for the fragmentation of **231**, **233** and **234** due to problems with reproducibility. The higher energy required for the fragmentation of the the oxazolidinones **210** and **235** compared to the isothiocyanate **231** are inconsistent with the results of the competition experiment discussed in section 4.4.3. The reasons for this inconsistency are not yet clear and are the subject of further investigations in the Crich laboratory (Figure 30).

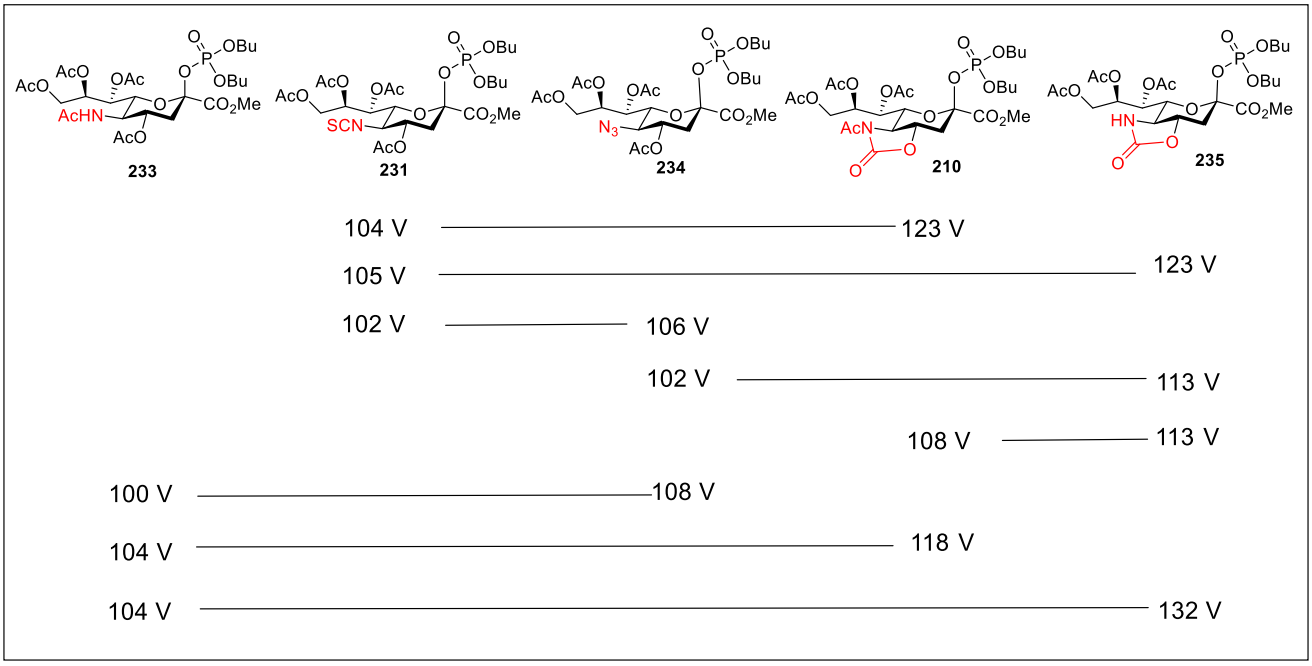


Figure 30: **Comparison of ESI cone voltages required to induce fragmentation of various sialyl phosphates**

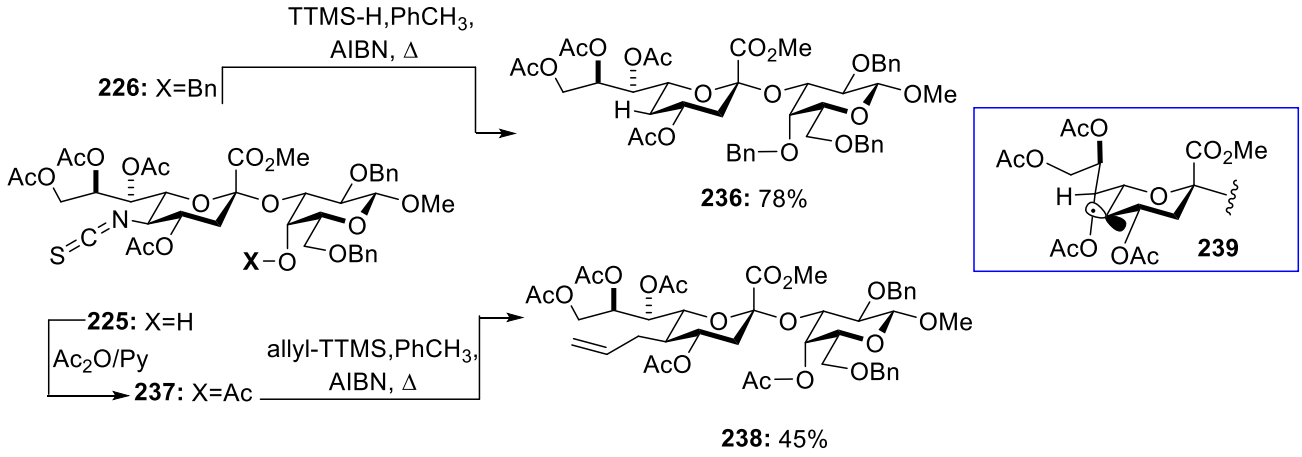
4.4.7. Post-glycosylation derivatization

The isothiocyanate group has very versatile chemistry and has found application in many areas of chemistry.[199,200] In view of the excellent selectivity of donor **218** in sialylation reactions, the adaptation of some of this chemistry promised to afford a range of sialosides carrying novel functional groups at C-5. The implementation of this idea is covered in the following section.

4.4.7.1. Radical deamination of sialyl glycosides

Radical deamination was achieved by treatment of the disaccharide **226** with tris(trimethylsilyl)silane and azobisisobutyronitrile (AIBN) in toluene at the reflux affording the 5-deamino-α-sialoside **236** in 78% yield (Scheme 43).[201,202] Further, acetylation of the residual alcohol in **225** followed by AIBN-initiated reaction with allyltris(trimethylsilyl)silane[203,204] in toluene at reflux gave the 5-deamino-5-allyl-α-sialoside **238** in 45% yield as a single isomer (Scheme 43). The selectivity observed in this radical reaction is consistent with that seen in the

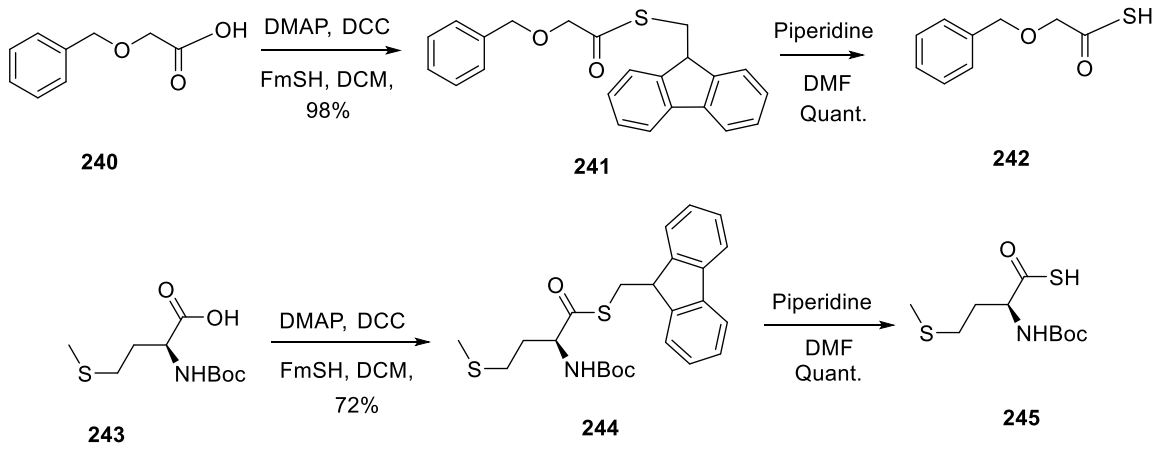
analogous C-4 glucopyranosyl radicals,[205,206] at and is a function of the face selectivity of radical **239**. As with conformationally locked cyclohexyl radicals[207] trapping of **239** occurs preferentially from the equatorial direction, presumably to avoid 1,3-diaxial interactions with the incoming radical trap. The equatorial preference for trapping of **239** is enhanced by the location on the axial face of the flanking substituents and especially by the conformation of the side chain[185] in which the 7-O-acetyl groups severely hinders approach from the axial direction.



Scheme 43: **Formation of desamino sialosyl disaccharides disaccharide and structure of radical 239**

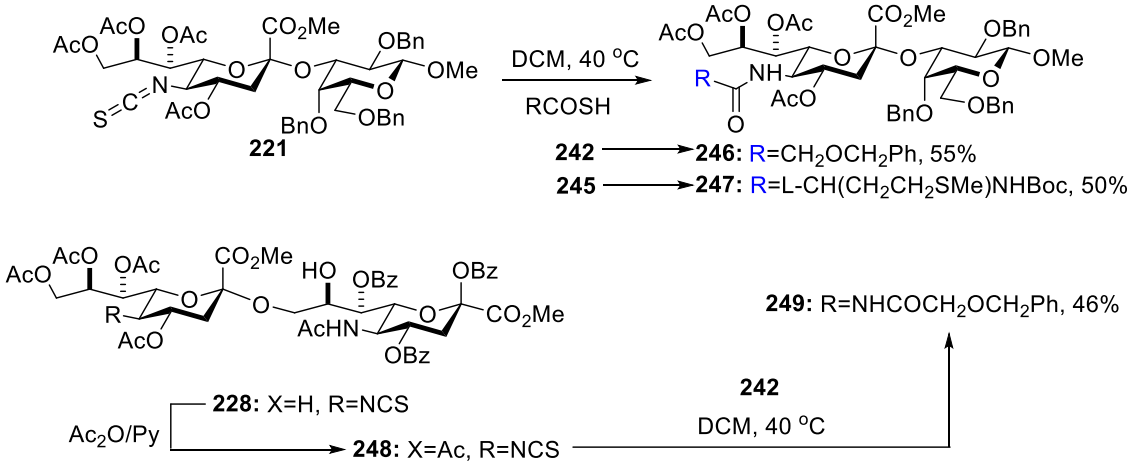
4.4.7.2. Transformation of the isothiocyanate to amides

The reaction of thioacids with isothiocyanates is known as a useful amide-forming reaction.[208] Thus, the reaction of isothiocyanato sialosides with thioacids was investigated. Two thioacids, **242** and **243** were prepared by the coupling reaction of corresponding acids with 9-fluorenylmethanethiol under standard carbodiimide conditions to give the 9-fluorenylmethyl thioesters **241** and **244,** respectively. Treatment of these thioesters with piperidine in DMF at room temperature gave the corresponding thioacids **242** and **245**, respectively (Scheme 44).[208,209]



Scheme 44: **Formation of thioacids**

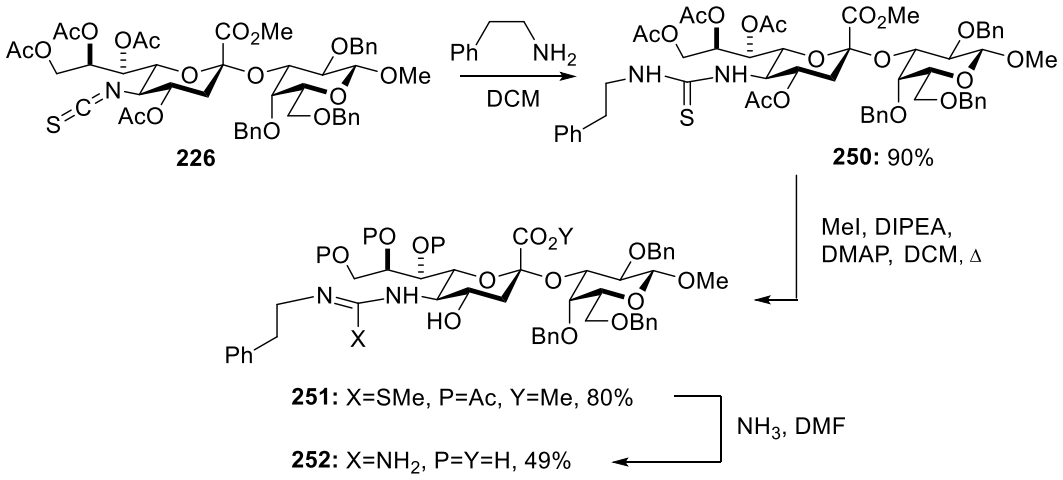
Then, the sialyl disaccharide **221** was reacted with the thioacids **242** and **245** in DCM at 40 ^{o}C leading to the isolation of the amides **246** and **247** in moderate yield. In a further example of the class the residual alcohol in the disialoside **228** was acetylated and the product **248** was allowed to react with benzyloxy thioacetic acid (**242**) in DCM at 40 ^{o}C to provide the disialoside **249** containing a protected glycolyl amide (Scheme 45).



Scheme 45: **Formation of amido derivatives from isothiocyanate sialosides**

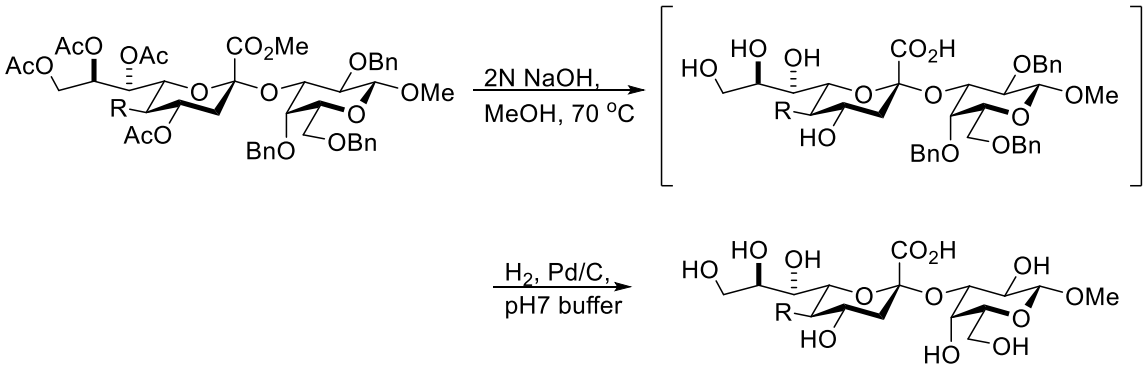
4.4.7.3. Synthesis of guanidine derivatives

In a further demonstration of the power of isothiocyanate chemistry the isothiocyanate-protected disaccharide **226** was converted to a guanidine group. In this sequence disaccharide **226** was treated first with 2-phenylethylamine to give the thiourea **250** in 90% yield. Subsequent reaction with methyl iodide gave an isothiourea **251**, which on treatment with ammonia in DMF at 130 ºC gave **252** in 49% yield (Scheme 46).



Scheme 46: **Synthesis of thiourea and guanidine derivatives**

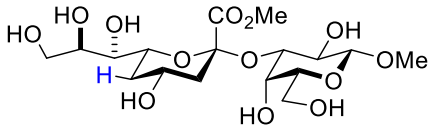
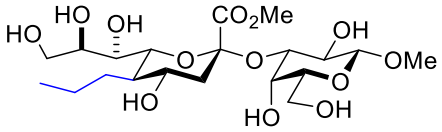
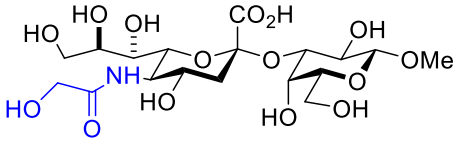
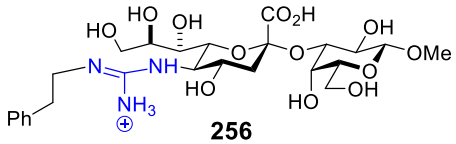
4.4.7.4. Deprotection of the sialosides



Scheme 47: **General scheme for deprotection of disaccharides**

A two-step protocol was developed for the deprotection of selected disaccharides. This approach involved the saponification of all esters followed by hydrogenolysis of benzyl ethers over palladium-charcoal in aqueous buffer (Scheme 47 and Table 11). It provides access to novel sialosides including those with a complete lack of substitution at the 5-position (**253**), or ones in which the acetamido function has been replaced by an alkyl chain (**254**), for the first time. In addition, a variety of C5 amides of the sialosides are accessible by this method, as illustrated by the *N*-glycoyl sialoside **255** and by the guanidine **256**.

Table 11: **Deprotection of selected disaccharides**

Entry	Substrate	Product	Yield
1	**236**	 **253**	91%
2	**238**	 **254**	93%
3	**242**	 **255**	91%
4	**252**	 **256**	52%

4.5. Conclusion

A crystalline sialyl donor (**218**) in which the nitrogen function is protected as an isothiocyanate has been prepared and demonstrated to be an excellent donor toward a variety of

primary and secondary glycosyl acceptors giving excellent selectivity and high yield in all cases. The corresponding sialyl phosphate **231** also gives excellent selectivity when used as a glycosyl donor, but yields are lower due to competing elimination. The presence of the isothiocyanate group facilitates direct introduction of a range of novel functionalities at the 5-position post-glycosylation.

CHAPTER 5. CONCLUSIONS

To improve the potential of apramycin as an antibiotic and resolve the uncertainty in its binding mode with bacterial and eukaryotic rRNA, a number of apramycin derivatives have been prepared by modifying the 6'- and *N*7'-positions. These derivatives were screened for antiribosomal activity in cell-free translation assays with a series of wild-type and mutant ribosomes, as well as for antibacterial activity against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli* (E coli). Unfortunately, all modifications of apramycin at 6' and 7' positions ended up with greater loss of activity against the bacterial wild-type ribosome than against the hybrid mutants of the human mitochondrial and cytosolic ribosomes. In particular, the modifications at the 6'-position including the inversion of the stereochemistry of 6'-hydroxy group, replacement of the 6'-hydroxy group by an amino group and complete removal of hydroxyl group had a significant influence on binding to the wild-type bacterial ribosomes. Thus, the inclusion and proper placement of a hydroxyl group at the 6'-position is significant for binding to the bacterial decoding A site. These results are consistent with apramycin adopting the standard binding mode of the 4,5- and 4,6-aminoglycosides in the decoding A site of the bacterial ribosome and are not in favor of the alternative binding mode proposed in some studies.

A series of novel paromomycin antibiotics were designed by focusing on the modification of ring I at the 4' and 6'-positions. The modifications consist of the introduction of an apramycin-like bicyclic scaffold containing a key hydroxy group or amine at the 6'-position to assist in binding to the ribosomal RNA. These newly designed paramomycin antibiotics were synthesized and screened for antiribosomal activity as well as for antibacterial activity. The bicyclic paromomycin derivative **155** with the equatorial 6’-hydroxy group displays better

activity against the bacterial wild-type ribosome than paromomycin itself; the epimer **154** with the axial hydroxyl group is significantly less active. The comparable amine derivatives are not as active as the parent paromomycin.

A novel sialyl donor with a highly electron withdrawing isothiocyanate functionality at the C-5 position (**218)** has been prepared and was demonstrated to be an excellent donor toward a variety of primary and secondary glycosyl acceptors giving outstanding stereoselectivity and high yields. The corresponding sialyl phosphate donor **231** also gives excellent selectivity in glycosylation reaction, but yields are lower due to competing elimination. The versatile isothiocyanate chemistry allows the isothiocyanate bearing saccharides to serve as precursors for the introduction of a range of novel functionalities at the 5-position post-glycosylation, which opens up access to new chemistry and potentially new biology at the 5-position of thesialyl glycosides.

CHAPTER 6. EXPERIMENTAL SECTION

General

All reagents and solvents were purchased from commercial suppliers and were used without further purification unless otherwise specified. All organic extracts were dried over sodium sulfate and concentrated under vacuum. Chromatographic purifications were carried out over silica gel, Sephadex G-10, Sephadex C-25 and Dowex 50WX8-100 sodium ion exchange resin. Analytical thin-layer chromatography was performed with pre-coated glass backed plates (w/UV 254) and visualized by UV irradiation (254 nm) or by staining with 25% H_2SO_4 in EtOH or ceric ammonium molybdate solution. Specific rotations were obtained using a digital polarimeter (Autopol III) in the solvent specified. High resolution mass spectra were recorded with an electrospray source coupled to a time-of-flight mass analyzer (Waters). ^{1}H, ^{13}C, ^{19}F, ^{31}P and 2D NMR spectra were recorded on 600 MHz (Agilent), 500 MHz and 400 MHz (Varian) instruments. Stereochemical assignments of coupled sialosides are based on $^{3}J_{C1\text{-}H3\text{-}ax}$ values. Ammonical methanol was prepared by using ammonium hydroxide solution (28% in water) and methanol in 1:9 ratio.

Chapter 2:

1,3,2′,4″-Tetraazidoapramycin (69) and 1,3,2′,4″,7'-Pentaazido-7'-demethylapramycin (70): Trifluoromethanesulfonyl azide was prepared fresh for each reaction as described here. Sodium azide (14.0 g, 0.21 mol) was dissolved in water (40.0 mL) and an equal volume of dichloromethane (40.0 mL) was added while stirring at room temperature. The resulting suspension was cooled to 0 $^{\circ}C$ and Tf_2O (30.0 g, 0.11 mol) was added drop wise over 45 min with vigorous stirring. The mixture was stirred at 0 $^{\circ}C$ for 3 h before sat. $NaHCO_3$ (45.0 mL) was added to quench the reaction. The organic layer was separated and the aqueous layer

was extracted with dichloromethane (10.0 mL). The organic layers were combined (triflyl azide solution) and kept at 0 °C until needed.

In a 500 mL round bottom flask, apramycin sulphate (**6**) (5.0 g, 0.0078 mol), $NaHCO_3$ (12.0 g, 0.142 mol) and $CuSO_4.5H_2O$ (0.3 g, 0.0013 mol) were dissolved in H_2O (50.0 mL) and cooled to 0 °C. Triflyl azide solution (freshly prepared dichloromethane solution) was added slowly to the reaction mixture at 0 °C over 0.5 h, followed by drop wise addition of MeOH (85.0 mL) over 0.5 h. The reaction mixture was allowed to come to room temperature and was stirred for 8 h before butylamine (1.2 g, 0.015 mmol) was added to quench the excess TfN_3. The solvent was evaporated under vacuum and the residue was purified by column chromatography over silica gel (eluent: gradient of 4% to 8% to 12% to 16% of ammonical methanol in dichloromethane) to give **69** (2.6 g, 50%) as a white solid and **70** (0.720 g, 15%) as a gum.

69: *Rf*= 0.47 (30% ammonical MeOH in EtOAc); mp: 112-114 °C; $[\alpha]_D^{25}$= +228.2 (*c*=0.93, MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ 5.59 (d, *J* = 3.3 Hz, 1H, H-1'), 5.28 (d, *J* = 3.7 Hz, 1H, H-1''), 4.87 (br s, 1H, H-8'), 4.20 (s, 1H, H-6'), 3.85-3.83 (dd, *J* = 9.9 Hz, 2.2 Hz, 1H, H-5'), 3.83–3.79 (m, 1H, H-3''), 3.79 (t, *J* = 3.7 Hz, 1H, H-4'), 3.78-3.74 (m, 2H, H-4'), 3.67-3.65 (dd, *J* = 4.4 Hz, 12.5 Hz, 2H, 6''-CH_2), 3.62-3.58 (m, 1H, H-5''), 3.52-3.47 (m, 3H, H-4, H-5, H-3), 3.47–3.45 (d, *J* = 3.7 Hz, 1H, H-2''), 3.42 (d, *J* = 4.4 Hz, 1H, H-1), 3.41-3.36 (t, 1H, H-4''), 3.24 (t, *J* = 9.2 Hz, 1H, H-6), 3.22-3.18 (dt, *J* = 12.8 Hz, 4.0 Hz, 1H, H-2'), 2.54 (dd, *J* = 2.6 Hz, 8.1 Hz, 1H, H-7'), 2.42 (s, 3H, N*CH*$_3$), 2.25-2.21 (dt, *J* =4.0 Hz, 12.8 Hz, 1H, H-2*ax*), 2.18-2.14 (dt, *J* =4.4 Hz, 11.4 Hz, 1H, H-3'*eq*), 2.00 (m, 1H, H-3'*ax*), 1.40 (m, 1H, H-2*eq*); ^{13}C NMR (151 MHz, CD_3OD): δ 97.6 (s, C-1′), 95.8 (s, C-8′), 94.5 (s, C-1′′), 79.2 (s, C-4), 76.6, 76.5 (s, C-6), 72.4 (s, C-3′′), 71.5 (s, CH), 71.1 (s, CH), 70.6 (s, C-5′), 66.4 (s, C-4′), 65.5 (s, C-6′′), 62.5 (s, C-7′), 62.0 (s, C-4′′), 60.9 (s, C-4), 60.3 (s, CH), 59.7 (s, CH), 56.4 (s, C-2′), 32.2 (s, N*CH*$_3$), 31.8

(s, C-2), 27.9 (s, C-3′′); ESI-HRMS: *m/z* calcd. for $C_{21}H_{34}N_{13}O_{11}$ $[M+H]^+$ 644.2501, found: 644.2501.

70: *Rf*= 0.49 (30% ammonical MeOH in EtOAc); $[\alpha]_D^{25}$= + 170.8 (*c*=6.6, MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ 5.59 (d, *J* = 3.3 Hz, 1H, H-1'), 5.30 (d, *J* = 3.7 Hz, 1H, H-1"), 5.05 (d, *J* = 8.4 Hz, 1H, H-8'), 4.20 (br s, 1H, H-6'), 3.87 (dd, *J* = 9.9 Hz, 2.2 Hz, 1H, H-5'), 3.85–3.70 (m, 4H, H-6, H-4', 6"-CH_2), 3.58 (dt, *J* = 10.3 Hz, 2.9 Hz, 1H, H-3"), 3.52-3.49 (m, 2H, H-4", H-2"), 3.49-3.43 (m, 3H, H-3, H-5, H-7'), 3.40 (dt, *J* = 4.4 Hz, 9.5 Hz, 1H, H-1), 3.29 (m, 1H, H-4), 3.27-3.20 (m, 2H, H-5", H-2'), 2.24 (dt, *J* = 4.4 Hz, 12.8 Hz, 1H, H-2*eq*), 2.18 (dt, *J* = 4.4 Hz, 11.0 Hz, 1H, H-3'*eq*), 2.17 (m, 1H, H-3'*ax*), 1.42 (m, 1H, H-2*eq*); ^{13}C NMR (151 MHz, CD_3OD): δ 97.58 (s, C-1′), 94.91 (s, C-1′′), 94.18 (s, C-8'), 79.26 (s, C-7"), 76.56 (s, C-2"), 76.49 (s, C-4), 72.34 (s, C-4'), 71.43 (2s, C-3", C-4"), 69.80 (s, C-5'), 68.02 (s, C-6'), 66.55 (s, C-6), 63.43 (s, C-5), 61.71, 60.68 (s, 6"-CH2), 60.31 (s, C-3), 59.70 (s, C-1), 56.35 (s, C-2'), 31.80 (s, C-2), 27.88 (s, C-3'); ESI-HRMS: *m/z* calcd. for $C_{20}H_{29}N_{15}O_{11}$ Na $[M+Na]^+$ 678.2069, found: 678.2059.

1,3,2′,4′′-Tetraazido-7′-*N*-benzyloxycarbonyl-apramycin (71): Sodium carbonate (3.1 g, 29.0 mmol) was added to a cold solution of **69** (3.76 g, 5.8 mmol) in 75% methanol in H_2O (75.0 mL). Benzyl chloroformate (3.0 g, 17.5 mmol) was added drop wise to the reaction mixture at 0 °C over 5 min, after which the reaction mixture was stirred at room temperature for 3 h. The solvent was evaporated under reduced pressure at room temperature and the residue was dried under reduced pressure for 1 h and then purified by column chromatography on silica gel (eluent: gradient of 2% to 4% to 6% to 8% to 10% MeOH in ethyl acetate) to give **71** (4.3 g, 93%) as a white solid. *Rf*= 0.5 (20% ammonical MeOH in EtOAc); mp: 116-119 °C; $[\alpha]_D^{25}$= +79 (*c*=0.8, MeOH); The ^{1}H-NMR spectrum showed the presence of two rotamers in a 4:3 ratio. ^{1}H NMR

(600 MHz, CD_3OD): δ 7.42–7.25 (m, 5H, ArHs), 5.60 (d, *J* = 3.3 Hz, 1H, H-1', major isomer), 5.54 (d, *J* =2.9 Hz,1H, H-1', minor isomer), 5.31 (d, *J* =8.8 Hz, 1H, H-8', major isomer), 5.28 (d, *J* =8.8 Hz, H-8', minor isomer), 5.24 (2br s, 1H, H-1'', 2 isomers), 5.17–5.06 (m, 2H, *CH*$_2$Ph), 4.19 (2br s, 1H, H-7'), 4.11 (m, 1H, H-5'), 3.94-3.83 (m, 2H, H-4', H-6'), 3.70-3.58 (m, 3H, 6''-*CH*$_2$, H-3''), 3.52-3.35 (m, 7H, H-3, H-4, H-4'', H-5'', H-5, H-6, H-2''), 3.25-3.16 (m, 2H, H-2', H-1), 3.12–3.03 (2br s, 3H, N*CH*$_3$), 2.29–2.14 (m, 2H, H-2*ax*, H-3'*eq*), 2.10–1.99 (m, 1H, H-3'*ax*), 1.45–1.35 (m, 1H, H-2*eq*); ^{13}C NMR (CD_3OD, 151 MHz): δ 157.71, 156.77 (2 s, C=O), 136.55, 136.42 (2 s arom.), 127.27, 127.43, 127.66, 127.69, 128.17, 128.20 (s, arom.), 97.73, 97.49, 97.42, 96.43, 95.06, 93.96 (s, C-1', C-1'', C-8'), 79.23, 79.05, 76.60, 76.54, 76.49, 72.48, 72.33, 71.62, 71.46, 70.39, 70.36, 69.91, 67.40, 67.19 (*CH*$_2$-Cbz); 66.53, 66.49, 61.82, 61.72, 60.79 (C-6''), 60.35, 60.31, 60.03, 59.65, 56.40 (C-2'), 31.82, 31.45 (s, N*CH*$_3$, C-3'), 28.00, 27.9 (C-2); ESI-HRMS: *m/z* calcd. for $C_{29}H_{39}N_{13}O_{13}Na$ $[M+Na]^+$ 800.2688, found: 800.2690.

1,3,2′,4′′-Tetraazido-6′,7′-oxazolidino-apramycin (72): Sodium hydride (433 mg, 60% in paraffin oil, 18 mmol) was added to an ice-cooled solution of **71** (4.2 g, 5.4 mmol) in dry DMF (15.0 mL) and stirred under Ar for 4 h during which the temperature was raised to room temperature. After completion, the reaction mixture was re-cooled to 0 ^oC and the pH adjusted to the neutral with 2 M HCl in ether. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography eluting with 5% ammonical methanol in dichloromethane to give **72** (3.14 g, 87%) as an off-white solid. *Rf*= 0.65 (5% ammonical MeOH in EtOAc); mp: 146-148 ^oC; $[\alpha]_D^{26}$ = +121.1 (*c*=0.7, MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ 5.33 (d, *J* = 3.3 Hz, 1H, H-1'), 5.28 (d, *J* = 3.7 Hz, 1H, H-1''), 5.11 (d, *J* = 2.6 Hz, 1H, H-8'), 4.94 (dd, *J* = 3.3 Hz, 10.6 Hz, 1H, H-5'), 4.87 (dd, *J* = 3.3 Hz, 8.8 Hz, 1H, H-6'), 4.02 (dd, *J* = 2.6 Hz, 8.8 Hz, 1H, H-7'), 3.80 (t, *J* = 9.6 Hz, 1H, H-3''), 3.75 (dd, *J* = 12.1 Hz, *J* = 2.9

Hz, 1H, H-4'), 3.76-3.63 (dd, *J* = 5.1 Hz, 12.1 Hz, 2H, 6"-CH_2), 3.59 (d, *J* = 4.4 Hz, 1H, H-3), 3.54 (dd, *J* = 3.7 Hz, 9.5 Hz, 1H, H-2"), 3.50-3.45 (m, 1H, H-5"), 3.46-3.31 (m, 4H, H-5, H-4, H-1, H-4"), 3.29 (t, *J* = 9.5 Hz, 1H, H-6), 3.23 (m, 1H, H-2'), 2.88 (s, 3H, N*CH*$_3$), 2.30 (m, 1H, H-2*eq*), 2.23 (m, 2H, H-3'*ax*,*eq*), 1.48 (q, *J* = 12.5 Hz, 1H, H-2*ax*); ^{13}C NMR (151 MHz, CD_3OD): δ 158.47 (s, C=O), δ 98.69 (s, C-1′), 94.62 (s, C-8′), 91.60 (s, C-1″), 81.14 (s, C-4), 76.32 (s, C-6), 72.52 (s, C-3″), 71.87 (CH), 71.31 (CH), 70.68 (s, C-5′), 65.53 (s, C-4′), 65.13 (s, C-6″), 62.27 (s, C-7′), 61.16 (s, C-4″), 60.34 (s, C-4), 60.06, 59.75, 58.49, 56.72 (s, C-2′), 31.29 (s, N*CH*$_3$), 29.60 (s, C-2), 28.63 (s, C-3″); ESI-HRMS: *m/z* calcd. for $C_{22}H_{31}N_{13}O_{12}$ Na $[M+Na]^+$ 692.2113, found: 692.2108.

1,3,2′,4″-Tetraazido-5,6,2″,3″,6″-penta-*O*-benzyl-6′,7′-oxazolidino-apramycin (73): To a stirred solution of compound **72** (3.15 g, 4.7 mmol) in DMF (65.0 mL) under Ar was added NaH (1.88 g, 60% in paraffin oil, 78.0 mmol) at 0 °C. After stirring for 0.5 h at the same temperature benzyl bromide (12.0 g, 70.6 mmol) was added drop wise at 0 °C and the resulting reaction mixture was stirred for 6 h at room temperature. On completion, the solvent was evaporated under reduced pressure at room temperature and the crude product was dissolved in EtOAc and washed sequentially with water and brine, dried, and concentrated under reduced pressure. Silica gel column chromatography of the residue eluting with EtOAc/toluene (2:8) gave **73** (4.8 g, 92%) as an off-white solid. *Rf*= 0.55 (20% EtOAc in toluene); mp: 152-154 °C; $[\alpha]_D^{26}$= +100.2 (*c*=1.0, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.42-7.20 (m, 25H arom.), 5.50 (d, *J* = 3.7 Hz, 1H, H-1'), 5.38 (d, *J* = 3.7 Hz, 1H, H-1"), 5.01-4.93 (m, 3H, 1 *CH*$_2$Ph and H-8'), 4.87-4.80, 4.79-4.66, 4.64-4.43 (m, 10H, 4 *CH*$_2$Ph, H-6', H-5'), 4.83 (br s, 1H, H-7'), 3.85–3.77 (m, 1H, H-4"), 3.72 (dt, *J* = 4.4 Hz, 11.0 Hz, 1H, H-4'), 3.67-3.55 (m, 5H, H-5", H-2", H-4, 6"-*CH*$_2$), 3.50-3.42 (m, 2H, H-5, H-3"), 3.35 (t, *J* = 9.5 Hz, 1H, H-6), 3.30 (dt, *J* = 4.4 Hz, 12.1 Hz, 1H, H-

1), 3.15 (dt, *J* = 4.0 Hz, 12.8 Hz, 1H, H-2'), 3.01(dt, *J* = 4.7 Hz, 12.8 Hz, 1H, H-3), 2.81 (s, 3H, N*CH*$_3$), 2.30 (m, 1H, H-3'*ax*), 2.21-2.09 (m, 2H, H-3'*eq*, H-2*eq*), 1.49-1.38 (q, *J* = 12.8 Hz, 1H, H-2*ax*,); ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.04 (s, C=O), 137.88, 137.59, 137.39, 137.23 (arom.), 128.52, 128.46, 128.39, 128.07, 127.99, 127.95, 127.91, 127.87, 127.60, 126.95 (arom.), 97.89 (s, C-1′), 94.50 (s, C-8′), 93.50 (s, C-1"), 84.64 (s, C-6), 84.00 (s, C-5), 79.69 (s, C-2"), 78.91 (s, C-3"), 78.28 (s, C-4), 75.81, 75.57, 74.94, 73.74, 73.07 (5s, 5PhCH_2), 70.97, 70.80 (s, C-6′), 68.78 (s, 6"-*CH*$_2$), 66.05 (s, C-5′), 65.52 (s, C-4′), 61.86, 60.06 (s, C-1), 60.05, 58.37 (s, C-3), 55.93 (s, C-2′), 31.78 (s, C-2), 30.08 (s, N*CH*$_3$), 29.34 (s, C-3′); ESI-HRMS: *m/z* calcd. for $C_{57}H_{61}N_{13}O_{12}Na$ $[M+Na]^+$ 1142.4460, found: 1142.4459.

1,3,2′,4″-Tetraazido-5,6,2″,3″,6″-penta-*O*-benzylapramycin (74): A stirred solution of **73** (4.8 g, 4.28 mmol) in 1,4-dioxane (77.0 mL) was treated with 3.0 M aqueous NaOH (40.0 mL) and heated to 100 °C for 13 h. The solvent was evaporated under reduced pressure and the residue taken up in ethyl acetate and washed with H_2O. The combined organic layers were washed with brine and concentrated under reduced pressure at room temperature. The residue was purified by column chromatography over silica gel, eluting with EtOAc:dichloromethane (3:7) to give **74** (3.65 g, 78%) as an off-white solid. *Rf*= 0.2 (30% EtOAc in toluene); mp: 68-70 °C; $[\alpha]_D^{26}$= +140.8 (*c*=0.93, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.42-7.22 (m, 25H arom.), 5.63 (d, *J* = 3.7 Hz, 1H, H-1'), 5.31 (d, *J* = 3.3 Hz, 1H, H-1"), 5.04-4.93 (m, 2H, H-6' and H-8'), 4.90-4.75, 4.72-4.65, 4.63-4.58, 4.52-4.44 (m, 10H, 5 *CH*$_2$Ph), 4.01 (d, *J* = 9.5 Hz, 1H, H-5'), 3.85–3.78 (m, 2H, H-4', H-4"), 3.74-3.56 (m, 8H, H-2", H-5", 6"-CH_2, H-4, H-5, H-6, H-7'), 3.51 (dt, *J* = 4.4 Hz, 9.5 Hz, 1H, H-3), 3.43-3.35 (m, 2H, H-1, H-3"), 3.16 (dt, *J* = 4.1 Hz, 13.2 Hz, 1H, H-2'), 2.74 (s, 1H, *NH*), 2.42 (br s, 3H, N*CH*$_3$), 2.33 (dt, *J* = 4.4 Hz, 13.2 Hz, 1H, H-2*eq*), 2.26-2.13 (m, 2H, H-3'*eq*, H-3*ax*), 1.49 (q, *J* = 12.8 Hz, 1H, H-2*ax*,); ^{13}C NMR (151 MHz,

$CDCl_3$): δ 137.97, 137.83, 137.42, 137.26, (4s, arom.), 128.46, 128.44, 128.39, 128.37, 128.25, 128.08, 128.00, 127.85, 127.79, 127.74, 127.62, 127.14 (arom.), 97.40 (s, C-1′), 94.0 (s, C-1"), 84.69 (s, C-3"), 84.50 (s, C-2"), 79.71 (s, C-4′), 78.63 (CH), 75.89, 75.70, 75.06, 73.56, 72.75 (5s, 5PhCH_2), 70.47, 70.20 (s, C-5′), 68.60 (s, 6"- *CH_2*), 66.44 (s, C-4"), 64.75, 62.78 (s, C-3"), 61.39 (s, C-5"), 60.25 (s, C-1), 60.04, 59.41 (s, C-3), 56.23, 56.14 (s, C-2′), 33.08 (s, N*CH_3*), 32.23(s, C-2), 28.20 (s, C-3′). ESI-HRMS: *m/z* calcd. for $C_{56}H_{64}N_{13}O_{11}$ $[M+H]^+$ 1094.4848, found: 1094.4813.

1,3,2′,4″-Tetraazido-5,6,2″,3″,6″-penta-*O*-benzyl-7′-*N*-benzylozycarbonyl-apramycin (75): Sodium carbonate (509 mg, 4.8 mmol) was added to a solution of **74** (1.05 g, 0.9 mmol) in methanol (30 mL) and cooled to 0 °C. Benzyl chloroformate (0.5 g, 2.9 mmol) was added drop wise to the reaction mixture at 0 °C over 5 min, after which the reaction mixture was stirred at room temperature for 3 h. The solvent was evaporated under reduced pressure at room temperature and the residue was purified by column chromatography on silica gel eluting with EtOAc:hexanes (5 to 40%) to give **75** (1.05 g, 90%) as a white solid. *Rf*= 0.3 (30% EtOAc in hexanes); mp:116-119 °C; $[\alpha]_D^{26}$= +126.4 (*c*=0.8, CH_2Cl_2); The ^{1}H-NMR spectrum showed the presence of two rotamers in a 3:2 ratio. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.38-7.25 (m, 30H arom.), 5.56-5.45 (m, 2H, H-1', H-1"), 5.29 (d, *J* = 3.8 Hz, 1H, H-8'), 5.24-4.38 (m, 12H, 5 *CH_2*Ph, H-5', H-4), 4.27 (br s, 1H, H-7'), 4.09 (d, *J* = 3.8 Hz, 1H, H-4', minor isomer), 4.06 (d, *J* = 9.5 Hz, 1H, H-4' major isomer), 3.93 (t, *J* = 10.6 Hz, 1H, H-6'), 3.83-3.30 (m, 8H, H-6', H-1, H-3, H-5, 6"-CH_2, H-3", H-4"), 3.16 (d, *J* = 3.3 Hz, 1H, H-2'), 3.03 (br s, 3H, N*CH_3*), 2.33 (m, 1H, H-2*eq*), 2.28-2.18 (m, 2H, H-3'*eq*, H-3'*ax*), 1.50 (m, 1H, H-2*ax*); ^{13}C NMR (151 MHz, $CDCl_3$): δ 158.8 (s, C=O), 138.03, 137.88, 138.67, 137.59, 137.26, 137.83, 137.42, 137.26, 136.6 (arom.), 128.54, 128.47, 128.37, 128.31, 128.20, 128.05, 127.95, 127.90, 127.78, 127.75,

127.71, 127.64, 127.27, 127.21 (arom.), 97.49 (s, C-1′), 96.4 (s, C-1''), 84.74, 84.38, 79.46, 78.74, 77.54, 75.88, 75.70, 75.63, 75.08, 73.54, 72.62 (5s, 5$PhCH_2$), 70.44, 68.16, 67.42, 66.47, 61.12, 60.22, 59.17, 56.22, 32.13 (s, NMe, C-2), 28.21(s, C-3′); ESI-HRMS: *m/z* calcd. for $C_{64}H_{73}N_{14}O_{13}$ $[M+NH4]^+$ 1245.5482, found: 1245.5428.

1,3,2′,4′′-Tetraazido-5,6,2′′,3′′,6′′-penta-*O*-benzyl-7′-*N*-benzyloxycarbonyl-6'-apramycinone (76): A solution of **75** (200.0 mg, 0.16 mmol) in dry dichloromethane (2.0 mL) was treated with Dess–Martin periodinane (103.0 mg, 0.24 mmol, 0.3 M solution in dichloromethane) and stirred for 8 h under Ar at room temperature. The reaction mixture was quenched by addition of saturated aqueous $NaHCO_3$, washed with brine, dried, and concentrated under reduced pressure. The crude product was charged on a silica gel column and eluted with EtOAc:hexanes (3:7) to afford the ketone **76** (178.0 mg, 90%) as a white foam. *Rf*= 0.65 (30% EtOAc and hexane); $[\alpha]_D^{26}$= +100.8 (*c*=0.8, CH_2Cl_2); The ^{1}H-NMR spectrum showed the presence of the two rotamers in 3:2 ration. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.50-7.14 (m, 30H arom.), 5.73 (d, *J* = 2.6 Hz, 1H, H-1', major isomer), 5.63 (br s, 1H, H-1', minor isomer), 5.28 (br s, 1H, H-1''), 5.12-4.32 (m, 12H, 5*CH*$_2$Ph, H-8', H-6'), 3.88-3.56 (m, 5H, H-3'', H-4'', H-5, H-6, H-4), 3.55-3.32 (m, 6H, H-2'', H-1, H-3, H-4, 6''-CH_2), 3.13 (br s, 3H, NMe), 3.09 (m, 1H, H-2'), 2.40 (m, 1H, H-3'*eq*), 2.34-2.29 (m, 2H, H-3'*ax*, H-2*eq*), 1.50 (q, *J* = 12.8 Hz, H-2*ax*); ^{13}C NMR (151 MHz, $CDCl_3$): δ 195.41 (br s, C=O), 155.85 (br s, C=O), 138.02-136.16 (arom.), 127.78-126.70 (arom.), 96.90 (s, C-1′), 94.41 (br s, C-8′, C-1''), 84.59, 84.45, 79.72, 79.62, 75.92-66.8 (5$PhCH_2$, C-6, 6''-*CH*$_2$), 61.16, 61.12, 60.27, 59.10, 55.76 (s, NMe), 55.56 (s, C-2'), 31.88 (s, C-2), 28.60 (s, C-3'); ESI-HRMS: *m/z* calcd. for $C_{64}H_{71}N_{14}O_{13}[M+NH_4]^+$ 1243.5325, found: 1243.5292.

1,3,2′,4′′-Tetraazido-5,6,2′′,3′′,6′′-penta-*O*-benzyl-7′-*N*-benzyloxycarbonyl-6′-*epi*-apramycin (77): Ketone **76** (100.0 mg, 0.08 mmol) was stirred with $NaBH_4$ (6.2 mg, 0.16 mmol) in methanol (4.0 mL) for 10 min. The reaction mixture was neutralized with 0.1 N HCl and concentrated under reduced pressure. The crude mixture of alcohols (5:1 ratio) was separated by silica gel column using 30% EtOAc in hexanes to give the title compound **77** (58.0 mg, 58%) as a white foam. *Rf*= 0.45 (30% EtOAc in hexanes); $[\alpha]_D^{25}$= +130.1 (*c*=0.8, CH_2Cl_2); The ^{1}H-NMR spectrum showed the presence of two rotamers in 3:2 ratio. ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.42–7.18 (m, 30H arom.), 5.51 (d, *J* = 2.9 Hz, 1H, H-1'), 5.24 (br s, 1H, H-1''), 5.11-4.34 (m, H-8', 5 *CH*$_2$Ph), 3.85-3.30 (m, 6H, H-6', H-1, H-3, H-5, H-3'', H-4''), 3.40 (m, 2H, 6''-*CH*$_2$), 3.30-2.82 (m, 3H, N*CH*$_3$), 3.11 (m, 1H, H-2'), 2.36-2.22 (m, 2H, H-2*ax*, H-3'*eq)*,1.54-1.25 (m, 2H, H-2*eq*, H-3'*ax*); ^{13}C NMR (151 MHz, $CDCl_3$): δ138.02, 137.60, 137.24, 136.21 (arom.), 128.46, 128.39, 128.29, 128.07, 128.00, 127.80, 127.71, 127.10 (arom.), 96.87-95.0 (s, C-1′, C-8′, C-1''), 84.73, 84.30, 79.71, 78.65, 78.00, 75.87, 75.67,74.99, 73.32, 72.28 (5s, 5Ph*CH*$_2$), 70.40, 70.20, 68.99, 68.55, 67.90, 67.72, 61.03, 60.20, 59.55, 56.41, 32.33 (s, C-2), 28.17 (s, N*CH*$_3$, C-3′); ESI-HRMS: *m/z* calcd. for $C_{64}H_{73}N_{13}O_{13}$ $[M+Na]^+$ 1250.5035, found: 1250.4999.

1,3,2′,4′′-Tetraazido-5,6,2′′,3′′,6′′-penta-*O*-benzyl-6'α-methyl-6′,7′-oxazolidino-apramycin (79): A stirred solution of **76** (100.0 mg, 0.081 mmol) in anhydrous diethyl ether (1.6 mL) under Ar was treated with freshly prepared methylmagnesium iodide (30.0 mg, 0.180 mmol, 2 M solution) at -20 ºC. The resulting reaction mixture stirred for 10 min and quenched with 1 N aqueous NH_4Cl. The organic layer was washed with 10% aqueous $Na_2S_2O_3$ followed by brine, dried, and concentrated under reduced pressure. The residue was purified by silica gel chromatography eluting with 2% to 30% EtOAc in hexanes to give the tertiary alcohol **78** (51.0 mg, 50%) as a thick oil. *Rf* = 0.32 (20% EtOAc in Hexanes); $[\alpha]_D^{26}$= +140.8 (*c*=0.8, CH_2Cl_2);

ESI-HRMS: *m/z* calcd. for $C_{65}H_{75}N_{14}O_{13}[M+NH_4]^+$ 1259.5638, found: 1259.5682. Compound **78** (7.0 mg, 0.005 mmol) in anhydrous DMF (0.2 mL) was treated with NaH (1.0 mg, 0.041 mmol) and stirred for 2 h at room temperature. The reaction mixture was extracted into EtOAc (1.0 mL) and washed with brine and dried over Na_2SO_4 and concentrated. The residue was purified by chromatography over silica gel eluting with ethyl acetate/hexanes (20% to 60%) to afford **79** (4.0 mg, 80%) as an oil. *Rf* = 0.48 (20% EtOAc in hexanes); $[\alpha]_D^{26}$= +185 (*c*=0.5, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.44-7.22 (m, 25H arom.), 5.54 (d, *J* = 3.7 Hz, 1H, H-1'), 5.41 (d, *J* = 3.7 Hz, 1H, H-1''), 5.04-4.99 (m, 3H, 1 *CH*$_2$Ph, H-8'), 4.85-4.68 (m, 10H, 4 *CH*$_2$Ph, H-5', H-6), 3.80 (t, *J* = 9.5 Hz, 1H, H-4''), 3.70-3.65 (m, 2H, H-4', H-2''), 3.63-3.55 (m, 3H, 6''-CH_2, H-3''), 3.50-3.40 (m, 2H, H-7', H-5''), 3.34 (t, *J* = 9.5 Hz, 1H, H-4), 3.26 (dt, *J* = 4.0 Hz, 9.5 Hz, 1H, H-3), 3.09 (dt, *J* = 4.0 Hz, 12.8 Hz, 1H, H-2'), 2.76 (m, 1H, H-1), 2.75 (s, 4H, H-5, N*CH*$_3$), 2.28 (dt, *J* = 4.4 Hz, 11.0 Hz, 1H, H-3'*eq*), 2.13-2.04 (m, 2H, H-3'*ax*, H-2*eq*), 1.56 (s, 3H, *CH*$_3$-6'), 1.38 (q, *J* = 12.8 Hz, H-2*ax*); ^{13}C NMR (151 MHz, $CDCl_3$): δ 156.49 (s, C=O), 137.96, 137.79, 137.37, 137.17 (arom.), 128.49, 128.45, 128.41, 128.13, 128.04, 127.98, 127.95, 127.92, 127.87, 127.77, 127.65, 127.06 (arom.), 97.47 (s, C-1′), 93.07 (s, C-8′), 92.92 (s, C-1''), 84.72 (s, C-4), 84.11 (s, C-7'), 79.83 (s, C-4''), 79.16 (s, C-4'), 78.17 (s, C-5), 75.87, 75.51, 74.89, 73.79, 72.95 (5s, 5PhCH_2), 70.73, 68.93 (s, C-5', 6''-*CH*$_2$), 66.24 (s, C-5''), 65.98 (s, C-2''), 62.08 (s, C-3''), 60.12 (s, C-3), 60.03, 57.75 (s, C-1), 55.86 (s, C-2'), 3.62 (s, C-2), 29.86 (s, N*CH*$_3$), 29.25 (s, C-3'), 23.84 (s, *CH*$_3$-6'); ESI-HRMS: *m/z* calcd. for $C_{58}H_{63}N_{13}O_{12}Na$ $[M+Na]^+$ 1156.4617, found: 1156.4604.

1,3,2′,4′′-Tetraazido-5,6,2′′,3′′,6′′-penta-*O*-benzyl-7′-*N*-benzyloxycarbonyl-6′-*O*-trifluoromethanesulfonyl-apramycin (80): To a stirred solution of **75** (600.0 mg, 0.49 mmol) in dry dichloromethane (5.0 mL) at room temperature was added diisopropylethylamine (152.0

mg, 1.18 mmol) in one portion. Triflic anhydride (304.0 mg, 1.078 mmol) was added to the reaction mixture at 0 ºC under Ar. The reaction mixture was stirred at 0 ºC for 1 h and was quenched with sat. $NaHCO_3$ solution and washed with brine, dried, filtered, and concentrated under reduced pressure. The crude product was purified via silica gel chromatography eluting with 5% to 25% EtOAc in hexanes to give **80** (424.0 mg, 64%) as a yellow foam. *Rf*= 0.6 (30% EtOAc in hexane); $[\alpha]_D^{26}$= +107.6 (*c*=0.7, CH_2Cl_2); The ^{1}H-NMR spectrum showed the presence of two rotamers in a 5:3 ratio. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.40-7.26 (m, 30H arom.), 5.52-5.32 (m, 3H, H-1', H-8', H-1''), 5.20-5.15, 5.04-4.27 (m, 12H, 5 *CH*$_2$Ph, H-6', H-5'), 3.80 (m, 1H, H-4'), 3.76-3.62 (m, 2H, H-5, H-6), 3.60-3.48 (m, 3H, 6''- *CH*$_2$, H-2''), 3.45-3.42 (m, 1H, H-1), 3.32 (dt, *J* = 4.8 Hz, 10.3 Hz, 1H, H-3), 3.10 (dt, *J* = 4.0 Hz, 12.5 Hz, 1H, H-2'), 2.98 (s, 3H, N*CH*$_3$,minor isomer), 2.95 (s, 3H, N*CH*$_3$, major isomer), 2.26-2.18 (m, 2H, H-3'*eq*, H-2*ex*), 1.59-1.42 (m, 2H, H-3'*ax*, H-2*eq*); ^{13}C NMR (151 MHz, $CDCl_3$): δ 156.3, 155.5 (s, C=O), 137.87, 137.56, 137.29, 136.11 (arom.), 128.55-127.08 (arom.), 121-117.5 (q, *J* = 319.8 Hz, CF_3), 97.58-95.51 (C-1′, C-8', C-1''), 87.46, 84.71, 83.93, 79.34, 78.75, 78.31, 75.88, 75.75, 75.04, 73.60, 73.15, 72.78 (5Ph*CH*$_2$, 6''-*CH*$_2$, H-2'', H-6') , 70.95, 68.12, 67.86, 66.91 (s, C-4'), 61.35, 60.24, 58.95, 57.0 (s, C-5'), 55.76 (s, C-2'), 31.82 (s, N*CH*$_3$), 29.68, 28.08 (s, C-2, C-3'); ^{19}F NMR (400 MHz, $CDCl_3$) δ -73.7; ESI-HRMS: *m/z* calcd. for $C_{64}H_{68}N_{13}O_{15}F_3SNa$ $[M+Na]^+$ 1382.4529, found: 1382.4501.

1,3,2′,4′′-Tetraazido-5,6,2′′,3′′,6′′-penta-*O*-benzyl-7′-*N*-benzyloxycarbonyl-6'-deoxy-6'α-iodo-apramycin (81): To a solution of **80** (220.0 mg, 0.162 mmol) in dry acetonitrile (3.0 mL) was added NaI (122.0 mg, 0.81 mmol) in one portion. The resulting reaction mixture was stirred at room temperature for 6 h and then concentrated under reduced pressure. The residue was dissolved in dichloromethane (2.0 mL) and washed with water and brine, and dried, and

filtered. After concentration under reduced pressure the crude product was purified via silica gel chromatography eluting with 2% to 20% EtOAc in hexanes to give **81** (158.0 mg, 80%) as an off white foam. *Rf* = 0.52 (20% EtOAc in hexanes); $[\alpha]_D^{26}$= +126.9 (*c*=1.3, CH_2Cl_2); The ^{1}H-NMR spectrum showed the presence of two rotamers in a 3:1 ratio. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.48-7.25 (m, 30H arom.), 5.70 (d, *J* =3.3 Hz, 1H, H-1'), 5.42 (d, *J* =8.1 Hz, 1H, H-8''), 5.24 (br s, 1H, H-1''), 5.2-4.4 (m, 14H, 5*CH*$_2$Ph, H-3'', H-4, H-5', H-6), 4.11 (t, *J* =9.5 Hz, 1H, H-5), 3.96 (t, *J* = 9.2 Hz, 1H, H-5'), 3.86 (m, 1H, H-6), 3.74 (m, 1H, H-6'), 3.6-3.4 (m, 4H, H-2'', H-4', H-1, H-3), 3.39 (m, 1H, H-7'), 3.17 (br s, 3H-N*CH*$_3$), 3.14 (dt, *J* =3.7 Hz, 12.5 Hz, 1H, H-2'), 2.38-2.19 (m, 3H, H-3'*eq*, H-2*ax*,H-2*eq*), 1.55 (m, 1H, H-3'*ax*); ^{13}C NMR (151 MHz, $CDCl_3$): δ 156.27, 154.98 (s, C=O), 137.98, 137.66, 137.40, 137.35, 136.13 (arom.), 128.92-127.38 (arom.), 96.29 (s, C-1'), 95.00 (s, C-8''), 93.32 (s, C-1''), 84.81 (s, C-4'), 84.64 (s, C-1), 79.59 (s, C-6'), 78.63 (s, C-4'), 75.90-56.62 (5Ph*CH*$_2$, C-4, C-3''), 70.34 (s, C-7'), 67.93 (s, C-6''), 60.56 (s, 6''-*CH*$_2$), 56.62 (s, C-2'), 40.29 (s, *CH*$_3$), 32.41 (s, C-3'), 27.96 (s, C-2); ESI-HRMS: *m/z* calcd. for $C_{64}H_{68}N_{13}O_{12}I$ Na $[M+Na]^+$ 1360.4053, found: 1360.4049.

1,3,2′,4′′-Tetraazido-5,6,2′′,3′′,6′′-penta-*O*-benzyl-6′α-trifluoromethyl-7′-*N* benzyloxycarbonylapramycin (84) and 1,3,2′,4′′-Tetraazido-5,6,2′′,3′′,6′′-penta-*O*-benzyl-6′β-trifluoromethyl7′-*N*-benzyloxycarbonylapramycin (85): To a stirred solution of **76** (120.0 mg, 0.097 mmol) under Ar in THF (3.0 mL) was added $TMSCF_3$ (280.0 mg, 1.97 mmol) followed by a catalytic amount of CsF (2.0 mg) in one portion at room temperature. The resulting reaction mixture was stirred for 1 h at room temperature and then concentrated to a afford gum. Purification by chromatography over silica gel eluting with EtOAc and hexanes (gradient of 2% to 20%) gave **84** (20.0 mg, 15%) as a white solid and **85** (60.0 mg, 50%) as a gum.

84: *Rf* = 0.66 (30% EtOAc in hexanes); $[\alpha]_D^{26}$= +112.9 (*c*=1.3, CH_2Cl_2); The ^{1}H-NMR spectrum showed the presence of two rotamers in a 3:2 ratio. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.39-7.23 (m, 30H arom.), 5.65 (d, *J* = 3.3 Hz, 1H, H-1', minor isomer), 5.65 (d, *J* = 3.3 Hz, 1H, H-1', major isomer), 5.22 (br s, 1H, H-1''), 5.20-4.35 (m, 13H, 5CH_2Ph, H-8', H-3'', H-4), 4.26 (t, *J* = 9.2 Hz, 1H, H-5'), 3.76 (t, *J* = 9.2 Hz, 1H, H-4''), 3.80-3.37 (m, 6H, 6''-CH_2, H-1, H-3, H-2'', H-3'') 3.99 (dt, *J* = 4.0 Hz, 12.8 Hz, 1H, H-2'), 2.97 (s, 3H, NCH_3, major isomer), 2.93 (s, 3H, NCH_3, minor isomer), 2.40-2.18 (m, H-2 *eq*, H-3'*eq*, H-3'*ax*), 1.47 (m, 1H, H-2*ax*), 0.14 (s, OTMS); ^{13}C NMR (151 MHz, $CDCl_3$): δ 156.53 (s, C=O), 138.10, 138.04, 137.88, 137.76, 137.56, 137.51, 137.30, 136.18 (arom.), 128.48-126.92 (arom.), 123.89 (q, *J* = 296.2 Hz, CF_3), 96.22-95.64 (m, C-1', C-1'', C-8'), 84.84, 84.73 (s, C-6'), 79.62, 79.23 (s, C-4''), 78.74, 75.98, 75.68, 75.61, 74.82, 73.61, 73.55, 72.69, 72.52, 70.50, 70.46, 68.86, 68.78 (s, C-5'), 68.52, 67.93, 67.83, 67.03 (5PhCH_2, 6''-CH_2), 61.21, 61.14, 60.41, 60.30, 58.76, 57.34, 55.89, 55.81 (s, C-2'), 32.09, 31.98, 31.89, 31.72 (s, C-2), 27.97 (s, C-3'), 2.35 (TMS); ESI-HRMS: *m/z* calcd. for $C_{68}H_{76}N_{13}O_{13}F_3SiNa$ [M+Na^+] 1390.5305, found: 1390.5298.

85: *Rf* = 0.7 (30% EtOAc in hexanes); $[\alpha]_D^{26}$=+109.3 (*c*=3.3, CH_2Cl_2); The ^{1}H-NMR spectrum showed the presence of two rotamers in 3:1 ratio. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.40-7.22 (m, 30H arom.), 6.03 (d, *J* = 8.2 Hz, 1H, H-8'), 5.70 (d, *J* = 3.4 Hz, H-1'), 5.46 (d, *J* = 3.4 Hz, H-1''), 5.23-4.38 (m, 14H, 5*CH*$_2$Ph, H-3'', H-4, 6''-CH_2), 4.23 (d, *J* = 10.7 Hz, 1H, H-4'), 4.08 (d, *J* = 10.7 Hz, 1H, H-5'), 3.83-3.46 (m, 4H, H-1, H-3, H-2'', H-3''), 3.32 (d, *J* = 8.8 Hz, 1H, H-7'), 3.18 (s, 3H, N*CH*$_3$), 3.06 (m, 1H, H-2'), 2.37 (m, 1H, H-2*eq*), 2.28-2.08 (m, 2H, H-3'*eq*, 3'*ax*), 1.41 (m, 1H, H-2*ax*), 0.31-0.08 (9H, OTMS); ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.32, 155.45 (s, C=O), 138.13, 137.87, 137.73, 137.22, 136.42 (arom.), 128.50, 128.47, 128.39, 128.36, 128.27, 128.17, 128.13, 128.05, 128.01, 127.93, 127.87, 127.79, 127.67, 127.41, 127.37,

127.30, 127.22 (arom.), 126.22-121.55 (4s, J = 289.5 Hz, CF_3), 96.58 (s, C-1'), 93.78 (s, C-1"), 92.53 (s, C-8"), 85.07 (s, C-6'), 84.44, 84.35, 84.27, 79.50, 78.81, 77.52, 76.77, 75.97, 75.43, 75.06, 74.85, 73.57, 73.47, 73.06, 72.18 (5PhCH_2), 70.71, 70.19, 68.08, 67.81, 66.90, 61.12, 60.48, 60.37, 60.19, 55.84, 42.21 (s, NCH_3), 32.92, 32.67 (s, C-2), 28.31 (s, C-3'), 2.29 (TMS); ESI-HRMS: *m/z* calcd. for $C_{68}H_{76}N_{13}O_{13}F_3SiNa$ $[M+Na]^+$ 1390.5305, found: 1390.5290.

1,3,2′,4″,6′-*epi*-Pentaazido-5,6,2″,3″,6″-penta-*O*-benzyl-7′-*N*-benzyloxycarbonyl-6'-deoxy- apramycin (88): To a solution of **80** (70.0 mg, 0.051 mmol) in dry DMF (1.2 mL) was added sodium azide (28.0 mg, 0.41 mmol) in one portion. The resulting reaction mixture was stirred at room temperature for 12 h after which the solvent was evaporated under reduced pressure. The residue was dissolved in dichloromethane (2.0 mL) and washed with water and brine, dried, filtered, and concentrated under reduced pressure. The crude product was purified via silica gel chromatography eluting with 2% to 20% EtOAc in hexanes to give **88** (36.0 mg, 55%) as an off white foam. *Rf* = 0.45 (20% EtOAc in Hexanes); $[\alpha]_D^{26}$= +125.7 (c=1.0, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.40-7.17 (m, 30H arom.), 5.58-5.41 (m, 2H, H-1', H-8'), 5.20 (br s, 1H, H-1"), 5.18-4.54 (m, 13H, 5CH_2Ph, H-3", H-4, H-6'), 4.03 (t, J = 9.2 Hz, 1H, H-5'), 3.75-3.43 (m, 6H, H-1, H-3, H-4',H-2', 6"-CH_2), 2.85 (br s, 1H, H-2"), 3.11 (m, 1H, H-2'), 3.08 (s, 3H, NCH_3), 2.35 (m, 1H, H-2 *eq*), 2.24-2.19 (m, 2H, H-3'*eq*, H-3'*ex*), 1.59-1.54 (q, J = 12.8 Hz, 1H, H-2*ax*); ^{13}C NMR (151 MHz, $CDCl_3$): δ 156.2, 155.06 (s, C=O), 137.98, 137.94, 137.85, 136.61,137.54 (arom.), 128.91-127.27 (arom.), 96.69 (s, C-1'), 95.10 (s, C-1"), 94.08 (s, C-8"), 84.77, 84.43, 79.57 (d, 6"-CH_2), 78.61, 77.72, 75.89, 75.66, 75.03, 73.55, 72.84, 71.92, 71.88 (5PhCH_2),70.53, 70.29, 69.80, 67.93, 67.10, 60.97, 60.39, 59.98, 59.22, 56.25 (s, C-7'), 39.74 (s, NCH_3), 32.12 (s, C-2), 28.11 (s, C-3'); ESI-HRMS: *m/z* calcd. for $C_{64}H_{68}N_{16}O_{12}Na$ $[M+Na]^+$ 1275.5100, found: 1275.5039.

1,3,2′,4′′-Tetraazido-5,6,2′′,3′′,6′′-penta-*O*-benzyl-7′-*N*-benzyloxycarbonyl-6′-*epi*-*O*-trifluoromethanesulfonyl-apramycin (89): To a solution of the 6'-epi alcohol **77** (210.0 mg, 0.171 mmol) in dry dichloromethane was added pyridine (42.0 mg, 0.53 mmol) at room temperature. Triflic anhydride (116.0 mg, 0.414 mmol) was added at 0 ºC under Ar and the reaction mixture was stirred at 0 ºC for 3 h. The reaction mixture was quenched with saturated aqueous $NaHCO_3$, and washed with brine, dried, filtered and concentrated under reduced pressure. The residue was purified via silica gel chromatography eluting with 4% to 30% EtOAc in hexanes to give **89** (195.0 mg, 84%) as a yellow foam. *Rf*= 0.6 (30% EtOAc in hexanes); $[\alpha]_D^{26}$= +97.3 (*c*=1.2, CH_2Cl_2). The ^{1}H-NMR spectrum showed the presence of two rotamers in a 5:2 ratio. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.45-7.25 (m, 30H arom.), 5.87 (t, *J* = 9.2 Hz, 1H, H-6'), 5.61-5.19 (m, 3H, H-1',H-8', H-1''), 5.10-4.49, 4.38-4.31 (m, 14H, 5 *CH*$_2$Ph, H-4'', H-5', H-6, H-5), 3.74-3.31 (m, 7H, H-4', H-1, H-3, 6''-CH_2, H-2'', H-3''), 3.26 (m, 1H, H-7'), 3.15 (m, 1H, H-2'), 3.06 (s, 3H, N*CH*$_3$), 2.35-2.23 (m, 3H, H-2*eq*, H-2*ex*, H-3'*eq*), 1.53 (m, 1H, H-3'*ax*); ^{13}C NMR (151 MHz, $CDCl_3$): δ 155.18 (s, C=O), 137.91, 137.88, 137.38, 137.26 (arom.), 128.82-126.98 (arom.), 121.59-115.2 (q, *J* = 320.3 Hz, CF3), 97.92-95.53 (C-1′, C-8', C-1''), 87.45 (s, C-6'), 84.41, 83.44, 79.71, 78.56, 75.90-67.58 (5Ph*CH*$_2$, 6''-*CH*$_2$, H-2'', H-6'), 66.54, 65.50, 61.91, 60.46, 58.62, 55.90 (s, C-2'), 39.27 (s, N*CH*$_3$), 31.77 (s, C-3'), 28.14 (s, C-2); ^{19}F NMR (400 MHz, $CDCl_3$) δ -75.0; ESI-HRMS: *m/z* calcd. for $C_{65}H_{68}N_{13}O_{15}F_3SNa$ $[M+Na]^+$ 1382.4529, found: 1382.4512.

1,3,2′,4′′,6′-Pentaazido-5,6,2′′,3′′,6′′-penta-*O*-benzyl-7′-*N*-benzyloxycarbonyl-6'-deoxy-apramycin (90): A solution of **89** (90.0 mg, 0.066 mmol) in dry DMF (1.2 mL) was treated with sodium azide (90.0 mg, 1.03 mmol) in one portion. The resulting reaction mixture was stirred at room temperature for 4 h before the solvent was evaporated under reduced

pressure. The residue was dissolved in dichloromethane (2.0 mL) and washed with water and brine, dried, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography eluting with 4% to 24% EtOAc in hexanes to give **90** (73.0 mg, 88%) as an off white foam. *Rf* = 0.65 (20% EtOAc in hexanes); $[\alpha]_D^{26}$=+157.4 (*c*=1.5, CH_2Cl_2); The ^{1}H-NMR spectrum showed the presence of two rotamers in a 1:1 ratio. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.40-7.25 (m, 30H arom.), 5.49 (2 br s, 1H, H-1', 2 isomers), 5.27 (2 br s, 1H, H-1", 2 isomers), 5.17 (m, 1H, H-8'), 5.2-4.4 (m, 14H, 5*CH*$_2$Ph, H-6', H-4, H-5', H-6), 4.13 (br s, 1H, H-7'), 3.80 (t, *J* =9.2 Hz, H-3"), 3.74 (dt, *J* =4.4 Hz, 10.3 Hz, H-4'), 3.70-3.49 (m, 7H, H-3", H-4", H-2", H-5, H-3, 6"-CH_2), 3.44 (t, *J* =9.2 Hz, 1H, H-1), 3.18 (dt, *J* =3.7 Hz, 12.5 Hz, H-2'), 3.02 (2 br s, 3H, NCH_3, 2 isomers), 2.35 (m, 1H, H-2 *eq*), 2.23-2.17 (m, 2H, H-3'*eq*, H-3'*ex*), 1.55 (m, 1H, H-2 *eq*); ^{13}C NMR (151 MHz, $CDCl_3$): δ 156.37, 156.06 (s, C=O), 138.05, 137.93, 137.69, 137.57, 137.30, 136.41 (arom.), 128.53-127.27 (arom.), 97.74, 97.60 (s, C-1'), 96.17-95.65 (s, C-1", C-8"), 84.80, 84.17 (s, C-1), 78.83, 78.17 (s, C-3"), 75.87, 75.68, 75.11 (s, C-2"), 73.60, 72.92, 72.59, 70.66, 69.79, 68.53, 68.46, 67.20 (5Ph*CH*$_2$), 67.77, 67.57 (s, 6"-*CH*$_2$), 63.67, 62.86 (s, C-7'), 61.31, 60.25 (s, C-3), 59.07 (s, C-1), 56.00 (s, C-2'), 32.03 (s, C-2), 31.37 (s, N*CH*$_3$), 28.22 (s, C-3'); ESI-HRMS: *m/z* calcd. for $C_{64}H_{68}N_{16}O_{12}Na$ $[M+Na]^+$ 1252.5100, found: 1252.5039.

1,3,2′,4″-Tetraazido-5,6,2″,3″,6″-penta-*O*-benzyl-7′-*N*-ethyl-apramycin (91): To a stirred solution of **74** (100.0 mg, 0.091 mmol) in anhydrous CH_2Cl_2 (4.0 mL) under Ar was added acetaldehyde (40.0 mg, 0.91 mmol) and $MgSO_4$ (50.0 mg) at room temperature. The resulting reaction mixture was stirred for 1 h then filtered through Celite. Sodium cyanoborohydride (30.0 mg, 0.476 mmol) was added to the filtrate and the reaction mixture stirred for 2 h before it was washed with water, and brine, dried, and concentrated to afford a colorless oil that was purified by flash chromatography over silica gel (eluent: 5% to 25% of

EtOAc/hexanes) to give **91** (76.0 mg, 74%) as a gum. *Rf* = 0.55 (50% EtOAc in hexanes); $[\alpha]_D^{26}$= +136.5 (*c*=2.0, dichloromethane); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.45-7.22 (m, 25H, arom.), 5.55 (d, *J* = 2.9 Hz, 1H, H-1'), 5.41 (d, *J* = 2.6 Hz, 1H, H-1''), 5.03 (d, *J* = 8.1 Hz, 1H, H-8'), 5.03-4.46 (m, 10H, 5*CH*$_2$Ph), 4.40 (br s, 1H, H-6'), 3.98 (d, *J* = 9.9 Hz, 1H, H-5'), 3.84 (m, 1H, H-3''), 3.78 (dt, *J* = 3.7 Hz, 10.6 Hz, 1H, H-4'), 3.68 (m, 3H, H-4, 6''-CH_2), 3.60 (m, 3H, H-3'', H-6, H-2''), 3.52 (dt, *J* = 4.0 Hz, 12.8 Hz, 1H, H-1), 3.42 (t, *J* = 9.5 Hz, 1H, H-5), 3.36 (dt, *J* = 4.0 Hz, 13.2 Hz, 1H, H-3), 3.12 (dt, *J* = 3.3 Hz, 12.8 Hz, 1H, H-2'), 2.80 (m, 3H, H-5'', 7'-N*CH*$_2$), 2.52 (br s, 3H, N*CH*$_3$), 2.34 (dt, *J* = 4.4 Hz, 13.2 Hz, 1H, H-2*eq*), 2.27-2.14 (m, 2H, H-3'*ax*, H-3'*eq*), 1.50 (m, 1H, H-2*ax*), 1.08 (br s, 3H, NCH$_2$*CH*$_3$); ^{13}C NMR (151 MHz, $CDCl_3$): δ 138.06, 137.91, 137.73, 137.69 (arom.), 128.48, 128.43, 128.40, 128.37, 128.33, 128.24, 128.05, 128.01, 127.93, 127.80, 127.78, 127.71, 127.67, 127.25, 127.11 (arom.), 97.57 (s, C-1′), 96.33 (s, C-8′), 94.59 (s, C-1''), 84.75 (s, C-5), 84.38 (s, C-6), 79.49, 78.79 (s, C-2''), 77.53 (s, C-4), 75.89, 75.58, 75.09, 73.59, 72.52 (5s, 5*CH*$_2$Ph), 70.83 (s, C-3''), 70.28 (s, C-5'), 69.22 (C-6'), 68.57 (s, 6''-*CH*$_2$), 66.08 (s, C-4'), 64.35, 61.44, 60.23 (s, C-1), 59.11 (s, C-3), 56.31 (s, C-2'), 50.20 (br s, 7'-N*CH*$_2$), 38.75 (s, N*CH*$_3$), 32.08 (s, C-2), 28.30 (s, C-3'), 13.19 (br s, NCH$_2$*CH*$_3$); ESI-HRMS: *m/z* calcd. for $C_{58}H_{68}N_{13}O_{11}$ $[M+H]^+$ 1122.5161, found: 1122.5130.

1,3,2′,4′′-Tetraazido-5,6,2′′,3′′,6′′-penta-*O*-benzyl-7′-*N*-ethyl-apramycin *N*-Oxide (92): *m*-CPBA (12.0 mg, 0.069 mmol) was added to the stirred solution of **91** (66.0 mg, 0.058 mmol) in CH_2Cl_2 (2 mL) and the reaction mixture was stirred for 1h at room temperature before it was washed with 1N aqueous NaOH, water, and brine. The dried organic layer was concentrated under vacuum to afford **92** (60.0 mg, 80%) as a white foam. *Rf* = 0.15 (60% EtOAc in hexanes). The ^{1}H-NMR spectrum showed the presence of two diastereomers in a 1:1 ratio. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.45-7.15 (m, 25H, arom.), 5.82, 5.78 (d, *J* = 7.7 Hz, 1H, H-8'),

5.68, 5.66 (d, *J* = 3.3 Hz, 1H, H-1'), 5.55, 5.53 (d, *J* = 3.7 Hz, 1H, H-1''), 5.03-4.42 (m, 11H, 5 CH_2Ph, H-6'), 3.98 (m, 1H, H-5), 3.89-3.48 (m, 8H, H-4', H-3'', H-4, H-5, H-5', H-4'', 6''-CH_2), 3.44-3.34 (m, 2H, H-1, H-3), 3.31, 3.24 (s, 3H, NCH_3), 3.19-3.11 (m, 2H, H-2',H-7'), 2.32 (m, 1H, H-3'*eq*), 2.25 (m, 1H, H-2*eq*), 2.11 (m, 1H, H-3'*ax*), 1.48 (m, 1H, H-2*ax*), 1.34-1.21 (m, 5H, NCH_2CH_3); ^{13}C NMR (151 MHz, $CDCl_3$): δ 137.81-137.14 (arom.), 128.62-126.90 (arom.), 97.53, 97.44 (s, C-1′), 93.34, 92.86 (s, C-8′), 92.72, 92.10 (s, C-1''), 84.63, 84.59, 84.44, 79.48, 78.38, 75.89-70.93 (5CH_2Ph), 70.73, 68.20, 67.04, 66.78, 66.56, 66.41, 60.20, 60.03, 59.66, 55.92, 55.05, 53.43, 32.41, 32.27 (s, C-2), 29.68 (m, NCH_2CH_3), 28.00, 27.88 (s, C-3'); ESI-HRMS: *m/z* calcd. for $C_{58}H_{68}N_{13}O_{12}$ $[M+H]^+$ 1138.5110, found: 1138.5092.

1,3,2′,4′′-Tetraazido-7′-*N*-demethyl-apramycin (94): To a stirred solution of **69** (110.0 mg, 0.171 mmol) in methanol (10.0 mL) was added sodium methoxide (120.0 mg, 2.22 mmol), tris base (270.0 mg, 2.23 mmol) and then iodine (130.0 mg, 0.51 mmol) at 0 ºC. The resulting reaction mixture was stirred for 3 h at 0 ºC, then warmed to room temperature and stirred for 12h. The solvent was evaporated under vacuum and the residue was purified by column chromatography on silica gel eluting with gradient of 2% to 20 % ammonical methanol in dichloromethane to give **94** (64.0 mg, 58%) as a thick gum. *Rf* = 0.2 (30% ammonical methanol in dichloromethane); $[\alpha]_D^{26}$= +90.7 (*c*=0.8, MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ 5.61 (d, *J* = 3.3 Hz, 1H, H-1'), 5.34 (d, *J* = 4.0 Hz, 1H, H-1''), 5.09 (d, *J* = 8.4 Hz, 1H, H-8'), 4.59 (br s, 2H, 7'-NH_2), 4.26 (t, *J* = 2.2 Hz, 1H, H-6'), 3.92-3.85 (m, 3H, H-5', 6''-CH_2), 3.79-3.64 (m, 4H, H-5, H-4', H-2'', H-3''), 3.57-3.44 (m, 4H, H-4'', H-5'', H-6, H-1), 3.44-3.37 (m, 2H, H-3'', H-3), 3.26-3.21 (m, 1H, H-2'), 2.26-2.18 (m, 2H, H-3'*eq*, H-2*eq*), 2.05 (m, 1H, H-3'*ax*), 1.48 (m, 1H, H-2*ax*); ^{13}C NMR (151 MHz, CD_3OD): δ 97.69 (s, C-1'), 94.57 (s, C-8'), 93.06 (s, C-1''), 79.34 (s, C-7') , 76.61, 76.45 (s, C-6'), 71.97, 71.52, 71.16 (s, C-2'), 69.81, 66.93, 66.09, 62.08, 61.08,

60.28, 59.65, 56.28, 53.77, 31.77 (s, C-2), 27.69 (s, C-3'); ESI-HRMS: *m/z* calcd. for $C_{20}H_{31}N_{13}O_{11}$ $[M+H]^+$ 630.2344, found: 630.2333.

1,3,2′,4″-Tetraazido-7'-*N*-(2-benzyloxyethyl)-7′-*N*-demethyl-apramycin (95): To a stirred solution of **94** (60.0 mg, 0.09 mmol) and 4 Å molecular sieves (50.0 mg) in anhydrous MeOH (2.0 mL) under Ar were added benzyloxyacetaldehyde (17.0 mg, 0.11 mmol), $NaBH_3CN$ (60.0 mg, 0.95 mmol) and 2 drops of glacial acetic acid. The reaction mixture was stirred for 2 h at room temperature then was filtered through Celite, and concentrated to afford a gum, which was purified by chromatography over silica gel eluting with gradient of 3% to 20% ammonical methanol in dichloromethane to give **95** (45.0 mg, 63%) as an off-white foam. $[\alpha]_D^{26}$= +57.2 (*c*=0.4, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.41-7.23 (m, 5H, arom.), 5.59 (d, *J* = 3.4 Hz, 1H, H-1'), 5.27 (d, *J* = 3.4 Hz, 1H, H-1''), 4.91 (d, *J* = 8.0 Hz, 1H, H-8'), 4.57-4.51 (m, 2H, 6''-*CH*$_2$), 4.17 (br s, 1H, H-6'), 3.84 (dd, *J* = 2.2 Hz, 9.9 Hz, 1H, H-7'), 3.82-3.67 (m, 4H, O*CH*$_2$, H-3'', H-4'), 3.57 (t, *J* = 4.8 Hz, 1H, H-4''), 3.52-3.43 (m, 5H, H-1, H-2'', H-4, H-6), 3.43-3.36 (m, 2H, H-3, H-5''), 3.23 (t, *J* = 9.2 Hz, 1H, H-5), 3.20 (dt, *J* = 4.3 Hz, 12.8 Hz, 1H, H-2'), 2.93 (br s, 2H, N*CH*$_2$), 2.70 (br s, 1H, H-5'), 2.24 (m, *J* = 3.7 Hz, 1H, H-2*eq*), 2.15 (m, 1H, H-3'*eq*), 2.01 (m, 1H, H-3'*ax*), 1.39 (m, 1H, H-2*ax*); ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.16 (s, arom.), 128.02, 127.60, 127.32 (3s, arom.), 97.64 (s, C-1'), 95.93 (s, C-8'), 94.89 (s, C-1''), 79.20 (s, C-6''), 76.60, 76.52, 72.85, 72.52, 71.46, 71.13, 70.43, 68.61, 66.41(s, N*CH*$_2$), 61.92, 60.80, 60.70, 60.31, 59.77, 56.42, 46.32(s, C-4'), 31.84 (s, C-2), 27.96 (s, C-3'); ESI-HRMS: *m/z* calcd. for $C_{29}H_{41}N_{13}O_{12}Na$ $[M+Na]^+$ 786.2896, found: 786.2872.

1,3,2',7'-Tetra-*N*-benzyloxycarbonyl-5,6-*O*-cyclohexylidene-epiaprosamine (99): To a stirred solution of **98**[101] (80.0 mg, 0.077 mmol) under Ar in anhydrous DCM (2.0 mL) was added Dess–Martin periodinane (98.0 mg, 0.23 mmol) and stirred for 8h under Ar at room

temperature. The reaction mixture was quenched by addition of saturated aqueous $NaHCO_3$, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to crude ketone. To a solution of this ketone in anhydrous MeOH (2.0 mL) under Ar was added $NaBH_4$ (5.8 mg, 3.89 mmol) and stirred for 10 min at room temperature. The reaction mixture concentrated under reduced pressure and crude dissolved in DCM (2.0 mL) was washed with water followed by brine. Then, the solvent was evaporated under reduced pressure and the crude mixture of alcohols (2:1 ratio) was separated by silica gel column using 30% EtOAc in hexanes to give the title compound **99** (40.0 mg, 41%) as a white foam. $[\alpha]_D^{25}$= +27.3 (*c*=1.2, CH_2Cl_2); The ^1H NMR spectrum showed the presence of two rotamers in 2:1 ratio. ^1H NMR ($CDCl_3$, 600 MHz): δ 7.30 (m, Ar-*H*), 6.18 (m, 2H, H-8'), 5.54 (d, *J* = 9.1 Hz, 2H, H-1'), 5.20-5.08 (m, 2H), 5.08-5.02 (m, 6H), 4.99 (m, 2H), 4.29 (s, 1H), 4.17-4.07 (m, 1H), 3.93-3.82 (m, 2H), 3.80 (s, 1H), 3.74 (m, 1H), 3.62 (d, *J* = 10.1 Hz, 1H), 3.53 (s, 1H), 3.45 (m, 1H), 3.32 (br s, 1H), 2.96 (s, 5H), 2.92 (s, 3H), 2.40 (s, 1H), 2.15 (m, 1H), 2.10 (s, 1H), 2.03 (br s, 3H), 1.96 (s, 5H), 1.72 (m, 1H), 1.52 (d, *J* = 7.3 Hz, 1H), 1.24 (q, *J* = 7.3 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.22, 156.89, 155.88, 155.67, 136.49, 136.28, 136.19, 128.52, 128.49, 128.46, 128.39, 128.15, 127.67, 113.07, 97.68, 89.37, 80.05, 77.25, 77.04, 76.83, 70.66, 70.37, 67.39, 66.89, 66.76, 51.68, 49.44, 36.25, 35.98, 35.61, 30.17, 24.75, 23.46, 20.89; ESI-HRMS: *m/z* calcd. for $C_{55}H_{64}N_4O_{16}Na$ $[M+Na]^+$ 1059.4215, found: 1059.4199.

General procedure for the Staudinger reduction of azides to amines: A stirred solution of substrate (0.06 mmol, 1 eq) in THF (5.0 mL) was treated with 0.1 M aqueous NaOH (0.3 mL, 0.03 mmol, 5 eq) and 1 M trimethylphosphine in THF (0.5 mL, 0.6 mmol, 10 eq) at room temperature. The reaction mixture was stirred for 2 h at 55 °C, and then cooled to room temperature, and neutralized with 1 M aqueous AcOH to pH 7 before concentration. The

resulting slurry was subjected to silica gel chromatography, eluting first with EtOAc (100 mL), followed by 20% of ammonical methanol in EtOAc (250 mL) to give the product.

General procedure for hydrogenolysis: The substrate (0.01 mmol) was dissolved in a mixture of methanol (1.0 mL), deionized water H_2O (1.0 mL), and glacial AcOH (10 eq). A catalytic amount of $Pd(OH)_2$ on carbon (20 wt. %) was added and the reaction mixture was stirred at room temperature under 1 atm of hydrogen (balloon) for 12-15 h. After completion, the reaction mixture was filtered through Celite® and the filtrate was neutralized by the addition of Amberlite-IRA400 to pH 7 and filtered. The filtrate was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography eluting with MeOH:H_2O:NH_4OH (1:0.4:0.4). The product containing fractions were evaporated and dissolved in 0.002 M aqueous AcOH (2.0 mL) and then charged to a Sephadex column (CM Sephadex C-25, 5.0 g). The Sephadex column was eluted with deionized water H_2O (50.0 mL), 0.5% aqueous NH_4OH (40.0 mL), and 1.5% NH_4OH (40.0 mL). The product-containing fractions were combined and evaporated to give the product in the form of the free base, which was taken up in H_2O (2.0 mL) and treated with glacial acetic acid (10 eq). The resulting solution was frozen in a dry ice acetone bath, and then lyophilized to give the product in the form of the acetate salt.

6'-epi-Aprosamine (19): A solution of compound **99** (40.0 mg, 0.04 mmol) in acetic acid/water (0.7 mL, 2:1) was heated at 60 ºC for 15 min. The mixture was diluted with EtOAc (2.0 mL) and washed with sat $NaHCO_3$ followed by brine. Then, the solvent was evaporated under reduced pressure and subjected to next reaction without further purification. The crude was dissolved in MeOH (1.0 mL) and sodium methoxide added at room temperature. The reaction mixture was stirred for 30 min and concentrated under reduced pressure. Then, reaction mixture dissolved in EtOAc (2.0 mL) and washed with 0.1N HCl, water, and followed by brine. The

volatiles were evaporated at 30 ºC *in vacuo* and the residue was dried under vacuum and was subjected to the hydrogenolysis using general procedure to give **19** (23.0 mg, 90%) as a white foam. $[\alpha]D^{26}$= +28.5 (*c*=0.13, H_2O); The ^1H NMR spectrum showed the presence of two anomers (8') in 5:1 ratio. ^1H NMR (600 MHz, D_2O) δ 5.31 (t, *J* = 2.9 Hz, 1H, 1'-H), 5.27 (m, 2H, 8'-H, 1'-H), 4.88 (d, *J* = 8.1 Hz, 1H), 3.83 (m, 1H), 3.78 (d, *J* = 6.9 Hz, 1H), 3.63 (t, *J* = 9.6 Hz, 1H), 3.50 (t, *J* = 11.7 Hz, 1H), 3.49-3.39 (m, 1H), 3.37-3.25 (m, 2H), 3.13-3.04 (br s, 1H), 2.60 (s, 1H), 2.57 (s, 3H), 2.25 (d, *J* = 12.5 Hz, 1H), 2.03(m, 1H), 1.74 (d, *J* = 12.8 Hz, 1H), 1.69 (s, 12H); ^{13}C NMR (151 MHz, D_2O) δ 181.14, 96.12, 92.11, 88.10, 81.37, 74.53, 72.36, 72.20, 66.26, 63.73, 61.19, 49.55, 48.77, 48.26, 30.83, 28.53, 27.23, 23.09; ESI-HRMS: *m/z* calcd. for $C_{15}H_{30}N_4O_7Na$ $[M+Na]^+$ 401.2012, found: 401.2026.

6'-Deoxyapramycin Pentaacetate Salt (107): Compound **82** (90.0 mg, 90%) was obtained in the form of a yellow thick gum by Staudinger reaction of **81** (95.0 mg) after silica gel chromatography (eluent: 20% ammonical methanol in EtOAc). $[\alpha]_D^{26}$ =+38.0 (*c*=2.5, MeOH); ESI-HRMS: *m/z* calcd. for $C_{64}H_{78}N_5O_{12}[M+H]^+$ 1234.4614, found: 1234.4611. This compound was taken forward to the next step without further characterization. To a solution of **82** (90.0 mg, 0.072 mmol) in toluene (3.0 mL) under Ar was added tris(trimethylsilyl)silane (0.1 g, 0.403 mmol) followed by azoisobutyronitrile (13.6 mg, 0.08 mmol) at room temperature. The resulting reaction mixture was stirred at 65 ºC for 3 h giving a mixture of the deiodinated product and its partially debenzylated congeners as determined by mass spectrometry. The solvent was evaporated under reduced pressure and the residue filtered on silica gel (eluent: 20% ammonical methanol in EtOAc) to give a mixture of **83** and partially debenzylated **83** (62.0 mg). This mixture (55.0 mg) was subjected to the standard hydrogenolysis protocol to give **107** (8.0 mg, 55%) as an off white foam after Sephadex chromatography. $[\alpha]_D^{26}$= +62.0 (*c* 0.3, H_2O); ^1H NMR

(600 MHz, D_2O): δ 5.47 (d, *J* = 3.7 Hz, 1H, H-1'), 5.38 (d, *J* = 3.3 Hz, 1H, H-1''), 4.88 (d, *J* = 8.4 Hz, 1H, H-8'), 3.82-3.59 (m, 6H, H-3'', H-5'', H-4, H-5', 6''-CH_2), 3.57-3.49 (m, 2H, H-2', H-2''), 3.45 (t, *J* = 9.2 Hz, 1H, H-5), 3.42-3.34 (m, 2H, H-4', H-6), 3.26 (t, *J* = 9.5 Hz, 1H, H-3), 3.20 (m, 1H, H-7'), 3.10 (m, 2H, H-1, H-4''), 2.58 (s, 3H, *N*Me), 2.43 (m, 1H, H-6'*eq*), 2.30 (m, 1H, H-2*eq*), 2.22 (m, 1H, H-3'*eq*), 1.80 (s, 15H, 5CH_3COOH), 1.89 (m, 1H, H-3'*ax*), 1.62-1.60 (m, 2H, H-2*ax*, H-6*ax*); ^{13}C NMR (151 MHz, D_2O): δ 178.76 (CO), 95.57 (s, C-8′), 95.52 (s, C-1'), 94.46 (s, C-1''), 78.49 (s, C-5'), 75.07 (s, C-5), 72.52 (s, C-4'), 72.24 (s, C-6), 70.23 (s, C-3'), 69.55, 67.00 (s, C-4), 60.33 (s, C-6''), 56.01 (s, C-7'), 52.11 (s, C-4''), 49.71 (s, C-1), 48.47 (s, C-3), 48.06 (s, C-2''), 30.20 (s, NMe), 28.41 (s, C-6'), 28.18 (s, C-2), 27.05 (s, C-3'), 21.73 (s, CH_3COOH); ESI-HRMS: *m/z* calcd. for $C_{21}H_{41}N_5O_{10}[M+H]^+$ 524.2932, found: 524.2909.

6'-epi-Apramycin Pentaacetate Salt (108): Compound **100** (30.0 mg, 85%) was obtained in the form of a white thick gum by Staudinger reaction of **77** (40.0 mg) after silica gel chromatography (eluent: 20% ammonical methanol in EtOAc). $[\alpha]_D^{26}$=+49.0 (*c*=0.8, MeOH); ESI-HRMS: *m/z* calcd. for $C_{64}H_{78}N_5O_{13}[M+H]^+$ 1124.5596, found: 1124.5450. This compound was taken forward to the next step without further characterization. Compound **107** (10.3 mg, 60%) was obtained in the form of a white foam by hydrogenolysis of **100** (27.0 mg) after Sephadex chromatography. $[\alpha]_D^{26}$=+51.0 (*c*=0.5, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.38–5.35 (m, 2H, H-1', H-1''), 4.98 (d, *J* = 8.4 Hz, 1H, H-8'), 3.86 (t, *J* = 9.2 Hz, 1H, H-6'), 3.83 (m, 1H, H-5''), 3.75–3.63 (m, 4H, H-3'', H-4'', 6''-CH_2), 3.61 (t, *J* = 9.2 Hz, 1H, H-5'), 3.58 (m, 1H, H-2''), 3.55 (t, *J* = 4.4 Hz, 1H, H-2'), 3.50 (t, *J* = 9.5 Hz, 1H, H-5), 3.42 (m, 2H, H-4', H-4), 3.32 (dt, *J* = 4.4 Hz, 12.1 Hz, 1H, H-3). 3.15 (dt, *J* = 4.4 Hz, 11.4, 1H, H-1), 3.10 (t, *J* = 10.3 Hz, 1H, H-6), 3.04 (t, *J* = 8.8 Hz, 1H, H-7'), 2.62 (s, 3H, N*CH*$_3$), 2.30 (dt, *J* = 4.0 Hz, 12.8 Hz, 1H, H-2*eq)*, 2.27-2.22 (m, 1H, H-3'*eq)*, 1.87 (m, 1H, H-3'*ax)*, 1.78 (s, 15 H, 5 CH_3COOH), 1.69-1.58

(m, 1H, H-2*ax*); ^{13}C NMR (151 MHz, D_2O): δ 180.49 (s, CO), 96.11 (s, C-1′), 95.02 (s, C-1′), 94.37 (s, C-1''), 81.32 (s, C-4'), 74.58 (s, C-5'), 72.36, 70.19, 70.02, 68.66, 68.26, 67.2 (s, C-6'), 66.93, 61.54 (s, C-7'), 60.22 (s, 6''-CH_2), 51.92, 49.55 (s, C-3), 48.79 (s, C-1), 48.08 (s, C-2'), 30.78 (s, NMe), 28.2 (s, C-2), 27.15 (s, C-3'), 22.72 (CH_3COOH); ESI-HRMS: *m/z* calcd. for $C_{21}H_{42}N_5O_{11}$ $[M+H]^+$ 540.2881, found: 540.2892.

6'α-Methylapramycin Pentaacetate Salt (109): Compound **78** (45.0 mg) was subjected to the Staudinger reaction using water instead of 0.1 N NaOH as solvent to give **101** (30.0 mg, 70%) in the form of a thick gum after silica gel chromatography (eluent: 20% ammonical methanol in EtOAc). $[\alpha]_D^{26}$=+79 (*c* 0.8, MeOH); ESI-HRMS: *m/z* calcd. for $C_{65}H_{80}N_5O_{13}[M+H]^+$ 1138.5753, found: 1138.5741. This compound was taken forward to the next step without further characterization. Compound **109** (12.0 mg, 75%) was obtained in the form of a white foam by hydrogenolysis of **101** (40 mg) after Sephadex chromatography. $[\alpha]_D^{26}$=+66.0.0 (*c*=0.5, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.55 (d, *J* = 2.8 Hz, 1H, H-1'), 5.40 (d, *J* = 3.1 Hz, 1H, H-1''), 5.11 (d, *J* = 8.2 Hz, 1H, H-8'), 3.85 (m, 1H, H-5''), 3.73 (m, 1H, H-4'), 3.70-3.62 (m, 4H, H-4'', H-4, 6''-*CH*$_2$), 3.57 (m, 2H, H-5', H-2''), 3.50 (m, 2H, H-2', H-6), 3.41 (t, *J* = 9.8 Hz, 1H, H-5), 3.27 (dt, *J* = 3.6 Hz, 11.3 Hz, 1H, H-1), 3.20-3.10 (m, 2H, H-3, H-3''), 3.02 (d, *J* = 8.2 Hz, 1H, H-7'), 2.77 (s, 3H, N*CH*$_3$), 2.29 (dt, *J* = 3.6 Hz, 12.5 Hz, 1H, H-2*eq*), 2.23 (dt, *J* = 3.6 Hz, 11.3 Hz, 1H, H-3'*eq*), 1.86 (m, 1H, H-3'*ax*), 1.78 (s, 15H, 5CH_3COOH), 1.67 (m, 1H, H-2*ax*), 1.37 (s, 3H, 6'-CH_3); ^{13}C NMR (151 MHz, D_2O): δ 180.86 (s, CO), 95.84 (s, C-1′), 95.09 (s, C-1''), 95.04 (s, C-8'), 80.43 (s, C-4''), 75.13 (s, C-2'), 72.55 (s, C-5), 70.84 (s, C-6'), 70.31 (s, C-5''), 70.01, 68.77 (s, C-5'), 66.63, 65.30 (s, C-7'), 60.31(s, 6''-*CH*$_2$), 52.01 (s, C-3''), 49.79 (s, C-3), 48.63 (s, C-1), 48.09 (s, C-6), 34.94 (s, N*CH*$_3$), 29.01 (s, C-2), 26.85 (s, C-3'), 22.93 (CH_3COOH), 20.52 (s, 6'-CH_3); ESI-HRMS: *m/z* calcd. for $C_{22}H_{44}N_5O_{11}$ $[M+H]^+$ 554.3037, found: 554.3054.

6'α-Trifluoromethylapramycin Pentaacetate Salt (110): To a solution of compound **84** (100.0 mg, 0.073mmol) in THF (2.0 mL) was added TBAF (38.0 mg, 0.15 mmol, 1M THF solution) at room temperature. The resulting reaction mixture was stirred for 1 h and then concentrated to afford a thick gum, which was purified by chromatography over silica gel (eluent: gradient of 4% to 30% EtOAc in hexanes) to give **86** (64.0 mg, 80%) as an off-white foam. This compound was taken forward to the next step without further characterization. Compound **86** (50.0 mg, 0.038 mmol) was subjected to the Staudinger reaction conditions to afford a 6′,7′-oxazolidinone in the form of a white thick gum from after silica gel chromatography (eluent: 20% ammonical methanol in EtOAc). This compound was taken up in 3 N NaOH (1.0 mL) and 1,4-dioxane (2.0 mL) and heated to 100 ºC for 6 h. After cooling to room temperature the reaction mixture was neutralized with glacial acetic acid followed by concentration to afford a thick mass that was purified by chromatography over silica gel (eluent: 20% ammonical methanol in EtOAc) to give the compound **102** (40.0 mg, 90%) as a gum. $[\alpha]_D^{26}$=+143 (*c* 0.7, MeOH); ESI-HRMS: *m/z* calcd. for $C_{65}H_{77}F_3N_5O_{13}[M+H]^+$ 1192.5470, found: 1192.5458. Compound **102** was taken forward to the next step without further characterization. Compound **110** (21.0 mg, 60%) was obtained in the form of a white foam by hydrogenolysis of **102** (50.0 mg) after Sephadex chromatography. $[\alpha]_D^{26}$=+77.0 (*c*=1.1, H_2O); ^1H NMR (600 MHz, D_2O): δ 5.50 (d, *J* = 3.7 Hz, 1H, H-1'), 5.30 (d, *J* = 3.7 Hz, 1H, H-1''), 4.99 (d, *J* = 6.6 Hz, 1H, H-8'), 4.29 (d, *J* = 9.9 Hz, 1H, H-5'), 3.84 (dt, *J* = 4.0 Hz, 10.3 Hz, 1H, H-4''), 3.75 (dd, *J* = 4.0 Hz, 11.4 Hz, 1H, H-4'), 3.71 (dd, *J* = 3.7 Hz, 12.5 Hz, 1H, H-3''), 3.68-3.60 (m, 3H, 6''-CH_2, H-4), 3.55 (dd, *J* = 3.7 Hz, 9.5 Hz, 1H, H-2''), 3.49 (dt, *J* = 4.0 Hz, 12.8 Hz, 1H, H-2'), 3.45 (t, *J* = 8.8 Hz, 1H, H-5), 3.39 (t, *J* = 9.9 Hz, 1H, H-6), 3.25 (dt, *J* = 4.0 Hz, 12.8 Hz, 1H, H-3), 3.17-3.08 (m, 3H, H-5'', H-7', H-1), 2.54 (s, 3H, N*CH*$_3$), 2.30-2.19 (m, 2H, H-2*eq*, H-

3'*eq*), 1.90 (q, *J* = 11.7 Hz, 1H, H-2*ax*), 1.76 (s, 15H, CH_3COOH), 1.67 (q, *J* = 12.8 Hz, H-3'*ax*); ^{13}C NMR (151 MHz, D_2O): δ 180.58 (s, CO), 127.40, 125.58, 123.67, 121.71 (q, *J* = 288.4 Hz, CF_3), 96.08 (s, C-1′), 95.71 (s, C-8'), 94.93 (s, C-1''), 80.95 (s, C-4), 75.11 (s, C-5), 72.30 (s, C-6), 70.42 (s, C-2''), 69.63, 68.93 (s, C-5''), 68.24 (s, C-5'), 65.33 (s, C-3''), 63.68, 60.70 (s, C-7'), 60.31 (s, 6''-CH_2), 52.11, 49.78 (s, C-1), 48.68 (s, C-3), 47.81 (s, C-2'), 35.08 (s, N*CH₃*), 28.78 (s, C-2), 27.05 (s, C-3'), 22.79 (s, CH_3COOH); ^{19}F NMR (400 MHz, $CDCl_3$) δ -75.6; ESI-HRMS: *m/z* calcd. for $C_{22}H_{41}F_3N_5O_{11}$ $[M+H]^+$ 608.2755, found: 608.2741.

6'-epi-6'β-Trifluoromethylapramycin Pentaacetate Salt (111): To a solution of compound **85** (60.0 mg, 0.043 mmol) in THF (2.0 mL) was added TBAF (23.0 mg, 0.087 mmol, 1M THF solution) at room temperature. The resulting reaction mixture was stirred for 1 h and concentrated to afford thick mass, which was purified by chromatography over silica gel (eluent: gradient of 4% to 30% EtOAc in hexanes) to give **87** (55.0 mg, 80%) as an off-white solid. This compound was taken forward to the next step without further characterization. Compound **103** (45.0 mg, 80%) was obtained in the form of a white thick gum by Staudinger reaction of **87** (50.0 mg) after silica gel chromatography (eluent: 20% ammonical methanol in EtOAc). $[\alpha]_D^{26}$=+153 (*c* 0.8, MeOH); ESI-HRMS: *m/z* calcd. for $C_{65}H_{77}F_3N_5O_{13}[M+H]^+$ 1192.5470, found: 1192.5446. Compound **103** was taken forward to the next step without further characterization. Compound **111** (20.6 mg, 72%) was obtained in the form of a white foam by hydrogenolysis of **103** (45.0 mg) after Sephadex chromatography. $[\alpha]_D^{26}$=+74.0 (*c*=1.1, H_2O); ^1H NMR (600 MHz, D_2O): δ 5.38 (d, *J* = 3.7 Hz, 1H, H-1'), 5.33 (d, *J* = 3.7 Hz, 1H, H-1''), 4.99 (d, *J* = 8.8 Hz, 1H, H-8'), 3.93 (d, *J* = 10.3 Hz, 1H, H-5'), 3.83 (m, 2H, H-5'', H-4'), 3.74 (t, *J* = 9.5 Hz, 1H, H-4), 3.72- 3.60 (m, 3H, 6''-CH_2, H-4''), 3.58-3.53 (m, 2H, H-2', H-2''), 3.47 (t, *J* = 9.2 Hz, 1H, H-5), 3.41 (m, 1H, H-6), 3.34 (dt, *J* = 4.0 Hz, 10.3 Hz, 1H, H-3), 3.17-3.08 (m, 2H, H-3'', H-1), 2.92 (d, *J* = 8.1

Hz, 1H, H-7'), 2.51 (s, 3H, N*CH*$_3$), 2.33 (dt, *J* = 4.0 Hz, 12.8 Hz, 1H, H-2*eq*), 2.24 (m, 1H, H-3'*eq*), 1.79 (m, 1H, H-3'*ax*), 1.77 (s, 15H, 5CH_3COOH), 1.67 (q, *J* = 12.5 Hz, 1H, H-2*ax*); ^{13}C NMR (151 MHz, D_2O): δ 180.80 (s, CO), 125.58 (q, *J* = 290.0 Hz, CF_3), 96.38 (s, C-1′), 95.81 (s, C-8'), 94.86 (s, C-1''), 81.88 (s, C-3''), 74.65 (s, C-6', C-5), 72.49 (s, C-6), 70.45 (s, C-2'), 69.80 (s, C-4'), 69.12 (s, C-4''), 67.19 (s, C-7'), 66.35 (s, C-5''), 60.32 (s, 6''-CH_2), 52.09 (s, C-1), 49.73, 48.74 (s, C-3), 47.76 (s, C-2''), 37.15 (s, N*CH*$_3$), 28.96 (s, C-2), 28.24 (s, C-3'), 22.92 (s, CH_3COOH); ^{19}F NMR (400 MHz, $CDCl_3$) δ -66.2; ESI-HRMS: *m/z* calcd. for $C_{22}H_{41}F_3N_5O_{11}$ $[M+H]^+$ 608.2755, found: 608.2746.

6'-*epi*-Amino-6'-deoxy-apramycin Hexaaacetate Salt (112): Compound **104** (30.0 mg, 85%) was obtained in the form of a white thick mass by Staudinger reaction of **88** (40.0 mg) after silica gel chromatography (eluent: 20% ammonical methanol in EtOAc). $[\alpha]_D^{26}$=+40 (*c* 0.7, MeOH); ESI-HRMS: *m/z* calcd. for $C_{64}H_{79}N_6O_{12}[M+H]^+$ 1123.5756, found: 1123.5747. Without further characterization **104** (30.0 mg) was subjected to the hydrogenolysis protocol to yield **112** (12.0 mg, 60%) in the form of a white foam after Sephadex chromatography. $[\alpha]_D^{26}$=+19.0 (*c*=1.2, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.58 (br s, 1H, H-1'), 5.32 (d, *J* = 3.7 Hz, 1H, H-1''), 4.92 (d, *J* = 8.1 Hz, 1H, H-8'), 3.85 (m, 1H, H-4''), 3.75-3.64 (m, 4H, H-3'', H-6, 6''-CH_2) 3.60–3.48 (m, 4H, H-2'', H-5, H-4', H-2'), 3.45–3.36 (m, 2H, H-5'', H-4), 3.23 (t, *J* = 9.5 Hz, 1H, H-1), 3.20-3.08 (m, 2H, H-6', H-3), 2.72 (t, *J* = 9.2 Hz, 1H, H-7'), 2.41 (s, 3H, N*CH*$_3$), 2.27 (m, 1H, H-2*eq*), 2.21 (m, 1H, H-3'*eq*), 1.92-1.86 (m, 1H, H-3'*ax*), 1.79 (s, 15H, 5CH_3COOH), 1.65-1.58 (q, *J* = 12.8 Hz, 1H, H-2*ax*); ^{13}C NMR (151 MHz, D_2O): δ 181(s, CO), 96.56 (s, C-8′), 95.03 (s, C-1''), 94.66 (s, C-1'), 79.06 (s, C-6), 75.13 (s, C-4'), 72.68 (s, C-4), 70.61, 70.36, 69.74 (s, C-4''), 69.29 (s, C-3''), 68.92, 60.64 (s, C-7'), 60.32 (s, 6''-CH_2), 60.03, 52.08 (s, C-3), 51.61 (s, C-6''),

48.53 (s, C-1), 48.05 (s, C-2'), 31.03 (s, N*CH*$_3$), 29.14 (s, C-2), 26.86 (s, C-3'), 22.95 (CH_3COOH); ESI-HRMS: *m/z* calcd. for $C_{21}H_{42}N_6O_{10}$ $[M+H]^+$ 539.3041, found: 539.3030.

6'-Amino-6'-deoxyapramycin Hexaacetate Salt (113): Compound **105** (44.0 mg, 76%) was obtained in the form of a thick mass form by Staudinger reaction of **90** (65.0 mg) after silica gel chromatography (eluent: 20% ammonical methanol in EtOAc). $[\alpha]_D^{26}$=+41 (*c* 0.8, MeOH); ESI-HRMS: *m/z* calcd. for $C_{64}H_{79}N_6O_{12}[M+H]^+$ 1123.5756, found 1123.5709. Without further characterization **105** (40.0 mg) was subjected to the standard hydrogenolysis protocol to give **113** (12.0 mg, 75%) in the form of a white foam after Sephadex chromatography. $[\alpha]_D^{26}$=+73.0 (*c*=0.6, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.50 (d, *J* = 3.7 Hz, H-1'), 5.32 (d, *J* = 3.7 Hz, 1H, H-1''), 4.95 (d, *J* = 8.8 Hz, 1H, H-8'), 3.82-3.68 (m, 6H, H-4'', H-5, H-4', H-6', 6''-CH_2), 3.65 (dd, *J* = 4.7 Hz, 12.5 Hz, 1H, H-5'), 3.62-3.55 (m, 2H, H-3'', H-5''), 3.52-3.45 (m, 2H, H-2', H-6), 3.39 (t, *J* = 9.9 Hz, 1H, H-4), 3.19 (dt, *J* = 3.7 Hz, 9.9 Hz, 1H, H-1), 3.13 (dt, *J* = 4.4 Hz, 12.5 Hz, 1H, H-3), 3.07-3.01 (m, 2H, H-7', H-2''), 2.49 (s, 3H, N*CH*$_3$), 2.27-2.19 (m, 2H, H-3'*eq*, H-2*eq*), 1.89 (m, 1H, H-3'*ax*), 1.77 (s, 18H, 6CH_3COOH), 1.60 (q, *J* = 12.5 Hz, 1H, H-2*ax*); ^{13}C NMR (151 MHz, D_2O): δ 180.8 (s, CO), 95.72 (s, C-1′), 94.40 (s, C-1''), 93.22 (s, C-8'), 80.11 (s, C-3''), 75.10 (s, C-6), 72.75 (s, C-4), 70.22 (s, C-6'), 69.88, 68.83, 68.70 (s, C-5), 65.94 (s, C-4''), 60.33 (s, 6''-CH_2), 59.66 (s, C-2''), 52.03 (s, C-7'), 49.85 (s, C-3), 48.41 (s, C-1), 47.93 (s, C-2'), 46.62, 30.98 (s, N*CH*$_3$), 29.41 (s, C-2), 27.11 (s, C-3'); ESI-HRMS: *m/z* calcd. for $C_{21}H_{42}N_6O_{10}$ $[M+H]^+$ 539.3041, found: 539.3045.

7'-*N*-Ethyl 7'-*N*-demethylapramycin Pentaacetate Salt (114): The *N*-oxide **92** (40.0 mg, 0.035 mmol) was dissolved in methanol (2.0 mL) and cooled in an ice-water bath followed by addition of a solution of $FeSO_4.7H_2O$ (20.0 mg, 0.071 mmol) in methanol (0.5 mL). This mixture was stirred at 0 °C for 6 h then basified to ~pH 10 with NH_4OH solution before an 1 M

aqueous EDTA (2 mL) was added. The reaction mixture was extracted with CH_2Cl_2, dried over Na_2SO_4, filtered and the solvent evaporated to afford a colorless thick mass that was purified by chromatography over silica gel (eluent: 40% EtOAc in hexanes) to yield compound **93** (14.0 mg, 40%). Without further characterization **93** (14.0 mg) was subjected to the Staudinger reaction to give **106** (9.0 mg, 71%) in the form of a thick mass after silica gel chromatography (eluent: 20% ammonical methanol in EtOAc). $[\alpha]_D^{26}$=+95 (*c* 0.6, MeOH); ESI-HRMS: *m/z* calcd. for $C_{57}H_{73}N_5O_{11}[M+H]^+$ 1004.5385, found: 1004.5370. Without further characterization **106** (40.0 mg) was subjected to the hydrogenolysis protocol to give **114** (2.0 mg, 40%) in the form of a white foam after Sephadex chromatography. $[\alpha]_D^{26}$=+47.0 (*c*=0.1, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.45 (d, *J* = 3.3 Hz, 1H, H-1'), 5.34 (d, *J* = 3.7 Hz, 1H, H-1''), 5.06 (d, *J* = 8.4 Hz, 1H, H-8'), 3.77 (dt, *J* = 4.0 Hz, 10.6 Hz, 1H, H-4'), 3.75-3.60 (m, 6H, H-4'', H-5'', H-6, H-5', 6''-CH_2), 3.55 (dd, *J* = 4.0 Hz, 9.9 Hz, 1H, H-2''), 3.53 (t, *J* = 9.5 Hz, 1H, H-5), 3.49-3.42 (m, 2H, H-2', H-3''), 3.34 (t, *J* = 9.9 Hz, 1H, H-4), 3.24 (dd, *J* = 2.2 Hz, 8.1 Hz, 1H, H-7'), 3.14-3.06 (m, 2H, NCH_2), 3.01 (t, *J* = 9.2 Hz, 1H, H-3), 2.95 (m, 1H, H-1), 1.86 (m, 2H, H-3'*eq*, H-2*eq*), 1.53 (m, 1H, H-3'*ax*), 1.75 (s, 15H, 6CH_3COOH), 1.53 (m, 1H, H-2*ax*), 1.13 (t, *J* = 9.9 Hz, 3H, NCH_2CH_3); ^{13}C NMR (151 MHz, D_2O): δ 180.87 (s, CO), 95.83 (s, C-1′), 94.55 (s, C-8'), 93.33 (s, C-1''), 75.19 (s, C-2'), 72.94 (s, C-4), 70.40 (s, C-3''), 70.27, 69.59, 69.27, 66.10 (s, C-4'), 63.42 (s, C-6'), 60.34 (s, 6''-CH_2), 58.08 (s, C-7'), 50.00 (s, C-3), 48.48, 48.06 (s, C-1), 40.23 (s, NCH_2), 34.21, 30.12 (s, C-2), 27.11 (s, C-3'), 23.10 (s, CH_3COOH), 22.94, 10.64 (s, CH_3); ESI-HRMS: *m/z* calcd. for $C_{22}H_{43}N_5O_{11}$ $[M+H]^+$ 554.3037, found: 554.3018.

7'-*N*-Demethylapramycin Pentaacetate Salt (**115**): Compound **70** (45.0 mg) was subjected to the Staudinger reaction using 0.1 N NaOH to give **115** (30.0 mg, 90%) in the form of a thick mass after silica gel chromatography (eluent: 20% ammonical methanol in EtOAc).

$[\alpha]_D^{26}$=+66.0 (*c*=1.3, H_2O); ^{1}H NMR (600 MHz, D_2O): δ 5.52 (d, *J* = 3.7 Hz, 1H, H-1'), 5.31 (d, *J* = 3.7 Hz, 1H, H-1''), 5.01 (d, *J* = 8.4 Hz, 1H, H-8'), 4.24 (br s, 1H, H-6'), 3.85-3.72 (m, 3H, H-4', H-3'', H-4''), 3.72-3.61 (m, 4H, H-5', H-6, 6''-*CH*$_2$), 3.54 (dd, *J* = 3.7 Hz, 9.5 Hz, 1H, H-2''), 3.48 (m, 2H, H-5, H-2'), 3.39 (t, *J* = 9.9 Hz, 1H, H-4), 3.30 (dt, *J* = 4.0 Hz, 10.6 Hz, 1H, H-3), 3.24 (dd, *J* = 2.9 Hz, 8.8 Hz, 1H, H-7'), 3.17-3.08 (m, 2H, H-1, H-5''), 2.30 (dt, *J* = 4.0 Hz, 12.5 Hz, 1H, H-2 *eq*), 2.20 (m, 1H, H-3'*eq*), 1.88 (m, 1H, H-3'*ax*), 1.75 (s, 15H, CH_3COOH), 1.67 (m, 1H, H-2*ax*); ^{13}C NMR (151 MHz, D_2O): δ 180.94 (s, CO), 95.67 (s, C-1′), 94.53 (s, C-1′′), 93.53 (s, C-8'), 79.26 (s, C-7'), 75.09 (s, C-2''), 72.62 (s, C-4), 70.32, 69.61, 69.47, 68.54, 66.10 (s, C-6'), 65.86, 60.36 (s, 6''-*CH*$_2$), 53.16 (s, C-5), 52.15 (s, C-3), 49.79 (s, C-1), 48.45 (s, C-3''), 47.99, 28.86 (s, C-2), 27.00 (s, C-3'), 23.01 (s, CH_3COOH); ESI-HRMS: *m/z* calcd. for $C_{20}H_{40}N_5O_{11}$ $[M+H]^+$ 526.2724, found: 526.2715.

7'-*N*-(2-Hydroxyethyl)-7'-*N*-demethyl-apramycin Pentaacetate Salt (117): Compound **116** (22.0 mg, 65%) was obtained in the form of a white thick mass by Staudinger reaction of **95** (40.0 mg) after silica gel chromatography (eluent: 20% ammonical methanol in EtOAc). $[\alpha]_D^{26}$=+132 (*c*=0.7, MeOH); ESI-HRMS: *m/z* calcd. for $C_{29}H_{50}N_5O_{12}$ $[M+H]^+$ 660.3456, found: 660.3451. Without further characterization **116** (27.0 mg) was subjected to the hydrogenolysis protocol to give **117** (14.6 mg, 75%) in the form of a white foam after Sephadex chromatography. $[\alpha]_D^{26}$=+83.0 (*c*=1.0, MeOH); ^{1}H NMR (600 MHz, D_2O): δ 5.52 (d, *J* = 3.3 Hz, 1H, H-1'), 5.33 (d, *J* = 4.0 Hz, 1H, H-1''), 5.04 (d, *J* = 8.4 Hz, 1H, H-8'), 4.35 (br s, 1H, H-6'), 3.86 (dt, *J* = 3.7 Hz, 10.6 Hz, 1H, H-5''), 3.78 (dt, *J* = 4.4 Hz, 11.0 Hz, 1H, H-4'), 3.81-3.60 (m, 6H, H-3'', H-4'', H-4, H-5', 6''-CH_2), 3.55 (dd, *J* = 3.7 Hz, 9.5 Hz, 1H, H-2''), 3.49 (m, 1H, H-5), 3.48 (t, *J* = 9.5 Hz, 1H, H-2'), 3.38 (t, *J* = 9.9 Hz, 2H, CH_2OH), 3.29 (dt, *J* = 3.7 Hz, 9.2 Hz, 1H, H-1), 3.17-3.05 (m, 4H, H-7', NCH_2, H-3), 2.98 (m, 1H, H-6), 2.28 (dt, *J* = 4.0 Hz, 8.4 Hz, 1H,

H-2*eq*), 2.19 (m, 1H, H-3'*eq*), 1.86 (m, 1H, H-3'*ax*), 1.76 (s, 15H, 5CH_3COOH), 1.64 (m, 1H, H-2*ax*); ^{13}C NMR (151 MHz, D_2O): δ 180.69 (s, CO), 95.66 (s, C-1′), 94.59 (s, C-1''), 94.15 (s, C-8'), 79.25 (s, C-6''), 75.09 (C-2'), 72.63 (s, CH_2OH), 70.32 (s, C-2''), 69.58 (s, C-5'), 68.71, 65.98 (s, NCH_2), 64.06 (s, C-6'), 60.28 (s, C-1), 60.03, 58.87 (s, C-7'), 57.70, 52.04 (s, C-7'), 49.77, 48.43 (s, C-3''), 47.99, 46.80 (s, C-4'), 28.90 (s, C-2), 26.97 (s, C-3'), 22.35 (s, CH_3COOH); ESI-HRMS: *m/z* calcd. for $C_{22}H_{44}N_5O_{11}[M+H]^+$ 570.2986, found: 570.2967.

Chapter 3:

6'-Allyl-1,3,2',2''',6'''-pentadeamino-1,3,2',2''',6'''-pentaazido-6,3',3'',5'',3''',4'''-hexa-O-benzyl-paromomycin (162*R* &162*S*): To a stirred solution of 1**60** (1.0 g, 0.77 mmol) under Ar in anhydrous DCM (20.0 mL) was added bis(acetoxy)iodobenzene (300.0 mg, 0.93 mmol) followed by a catalytic amount of TEMPO (12.0 mg, 0.08 mmol) in one portion at room temperature. The resulting reaction mixture was stirred for 12 h at room temperature and was quenched with sat. $Na_2S_2O_3$ solution, washed with sat. $NaHCO_3$, brine, dried over Na_2SO_4, and concentrated under reduced pressure to give crude aldehyde **161**. To a solution of this aldehyde in anhydrous DCM (10.0 mL) under Ar was added allyltributyltin (1.29 g, 3.89 mmol) followed by boron trifluoride ethyl etherate (133.0 mg, 0.04 mmol) at 0 ^oC. The resulting reaction mixture was stirred at 0 ^oC for 2 h before it was quenched with sat. $NaHCO_3$. The comibined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated to a afford gum. The crude product was purified via silica gel column chromatography (eluent: 2%-30% EtOAc in hexane) to give **162*R*** (295 mg, 30%, over 2 steps) and **162*S*** (293 mg, 28%, over 2 steps), as a yellow foam.

Compound 162*R*: $[\alpha]_D^{26}$= +82.5 (*c*=0.20, CH_2Cl_2); ^1H NMR (600 MHz, Chloroform-*d*): δ 7.46-7.18 (m, 30H, Ar-*H*), 6.21 (d, *J* = 3.6 Hz, 1H, H-1'), 5.97 (m, 1H, 8'-CH), 5.74 (d, *J* = 5.7

Hz, 1H, H-1''), 5.29-5.21 (m, 2H, H-9'*CH*$_2$), 5.03 (d, *J* = 10.6 Hz, 1H, *CH*$_2$Ph), 4.96 (d, *J* = 1.9 Hz, 1H, H-1'''), 4.92 (d, *J* = 11.1 Hz, 1H, *CH*$_2$Ph), 4.84 (d, *J* = 11.2 Hz, 1H, *CH*$_2$Ph), 4.77 (d, *J* = 10.6 Hz, 1H, *CH*$_2$Ph), 4.67 (d, *J* = 12.0 Hz, 1H, *CH*$_2$Ph), 4.62 (d, *J* = 11.8 Hz, 1H, *CH*$_2$Ph), 4.60-4.42 (m, 4H, *CH*$_2$Ph), 4.39-4.33 (m, 3H, H-3'', H-4'', *CH*$_2$Ph), 4.30 (d, *J* = 12.1 Hz, 1H, *CH*$_2$Ph), 4.05-3.94 (m, 3H, H-2'', H-5, H-3'), 3.90-3.75 (m, 5H, H-5', H-3''', H-6', H-5''*CH*$_2$,OH), 3.71 (dd, *J* = 13.0, 8.6 Hz, 1H, H-6'''*CH*$_2$), 3.65 (t, *J* = 9.4 Hz, 1H, H-4), 3.62 (dd, *J* = 2.2, 1.8 Hz, 1H, H-5'' *CH*$_2$), 3.52 (t, *J* = 9.0 Hz, 1H, H-4'), 3.50-3.42 (m, 2H, H-1, H-3), 3.41 (br s, 1H, H-2'''), 3.32 (t, *J* = 9.3 Hz, 1H, H-6), 3.27 (br s, 1H, H-5'''), 3.17 (t, *J* = 2.3 Hz, 1H, H-4'''), 2.95 (dd, *J* = 10.3, 3.7 Hz, 1H, H-2'), 2.92 (dd, *J* = 13.0, 3.8 Hz, 1H, H-6'''*CH*$_2$), 2.77 (br s, 1H, OH), 2.61 (dt, *J* = 15.0, 4.0 Hz, 1H, H-7'*CH*$_2$), 2.33 (dt, *J* = 15.1, 8.0 Hz, 1H, H-7'*CH*$_2$), 2.24 (dt, *J* = 13.2, 4.6 Hz, 1H, H-2*CH*$_2$), 1.41 (q, *J* = 12.7 Hz, 1H, H-2*CH*$_2$); ^{13}C NMR (151 MHz, Chloroform-*d*): δ 138.31, 138.23, 137.97, 137.57, 137.04, 136.98 (Ar-*C*), 134.62 (C-8'), 128.72, 128.57, 128.56, 128.46, 128.40, 128.38, 128.34, 128.32, 128.24, 128.21, 127.95, 127.87, 127.80, 127.55, 127.50, 127.24 (arom.), 118.48 (C-9'), 106.18 (C-1''), 98.67 (C-1'''), 95.60 (C-1'), 84.36 (C-6), 82.48 (C-2''), 82.21(C-5), 81.92 (C-4''), 79.24 (C-3'), 75.55 (C-3''), 75.10 (C- *CH*$_2$Ph, C-4), 75.03 (C-*CH*$_2$Ph), 74.51 (C-*CH*$_2$Ph), 74.49 (C-4'), 73.59 (C-6'), 73.26 (C-*CH*$_2$Ph), 73.22 (C-5'), 72.92 (C-3'''), 72.42 (C-4'''), 71.85 (C-*CH*$_2$Ph), 71.76 (C-*CH*$_2$Ph), 71.55 (C-5'''), 70.33 (C-5''), 62.39 (C-2'), 60.41 (C-1), 60.33 (C-3), 57.29 (C-2'''), 51.18 (C-6'''), 37.74 (C-7'), 32.67 (C-2); ESI-HRMS: *m/z* calcd. for $C_{68}H_{75}N_{15}O_{14}Na$ $[M+Na]^+$ 1348.5516, found: 1348.5491.

Compound 162*S*: $[\alpha]_D^{26}$= +58.8 (*c*=0.27, CH_2Cl_2); ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.42-7.13 (m, 30H, Ar-H), 6.19 (d, *J* = 3.6 Hz, 1H, H-1'), 5.91 (m, 1H, H-8'), 5.72 (d, *J* = 5.9 Hz, 1H, H-1''), 5.21-5.11 (m, 2H, H-9'), 5.01 (d, *J* = 10.5 Hz, 1H, *CH*$_2$Ph), 4.96-4.90 (m, 2H, H-1''', *CH*$_2$Ph), 4.73 (d, *J* = 10.5 Hz, 1H, *CH*$_2$Ph), 4.70 (d, *J* = 11.4 Hz, 1H, *CH*$_2$Ph), 4.62 (m, 2H,

CH$_2$Ph), 4.54-4.39 (m, 4H, *CH*$_2$Ph), 4.33 (m, 3H, *CH*$_2$Ph, H-3", H-4"), 4.26 (d, *J* = 12.0 Hz, 1H, *CH*$_2$Ph), 4.01 (dd, *J* = 5.9, 4.8 Hz, 1H, H-2"), 3.97 (t, *J* = 8.9 Hz, 1H, H-5), 3.92-3.83 (m, 3H, H-3', H-6', H-5"*CH*$_2$), 3.81 (m, 1H, H-5'"), 3.79-3.75 (m, 2H, H-5', H-3'"), 3.67 (dd, *J* = 13.0, 8.7 Hz, 1H, H-6'"*CH*$_2$), 3.62-3.55 (m, 2H, H-4, H-5"*CH*$_2$), 3.52 (t, *J* = 9.4 Hz, 1H, H-4'), 3.45 (m, 2H, H-1, H-3), 3.38 (t, *J* = 2.3 Hz, 1H, H-2'"), 3.29 (t, *J* = 9.4 Hz, 1H, H-6), 3.13 (d, *J* = 2.4 Hz, 1H, H-4'"), 2.88 (dd, *J* = 13.0, 3.8 Hz, 1H, H-6'"*CH*$_2$), 2.83 (dd, *J* = 10.3, 3.6 Hz, 1H, H-2'), 2.43-2.34 (m, 1H, H-7'*CH*$_2$), 2.34-2.26 (m, 1H, H-7'*CH*$_2$), 2.23 (dt, *J* = 13.2, 4.6 Hz, 1H, H-2*CH*$_2$), 1.35 (q, *J* = 12.8 Hz, 1H, H-2*CH*$_2$); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 138.26, 138.12, 137.89, 137.53, 136.98, 136.92 (arom.), 135.07 (C-8'), 128.67, 128.63, 128.51, 128.41, 128.37, 128.33, 128.27, 128.20, 128.11, 128.03, 127.81, 127.80, 127.68, 127.58, 127.51, 127.02 (arom.), 117.48 (C-9'), 106.10 (C-1"), 98.69 (C-1'"), 95.93 (C-1'), 84.42 (C-6), 82.48 (C-2"), 82.20 (C-4"), 81.93 (C-5), 79.68 (C-3'), 75.51 (C-3'"), 75.13 (C-*CH*$_2$Ph), 74.93 (C-*CH*$_2$Ph), 74.81 (C-4), 74.45 (C-5'"), 73.12 (2C-*CH*$_2$Ph), 72.86 (C-4'"), 72.58 (C-3'"), 72.37 (C-5'), 71.71 (C-*CH*$_2$Ph), 71.47 (C-*CH*$_2$Ph), 70.21 (C-5"*CH*$_2$), 70.04 (C-4'), 68.73 (C-6'), 62.48 (C-2'), 60.40 (C-1), 60.33 (C-3), 57.25 (C-2'"), 51.14 (C-6"), 38.48 (C-7'), 32.70 (C-2); ESI-HRMS: *m/z* calcd. for $C_{68}H_{75}N_{15}O_{14}Na$ $[M+Na]^+$ 1348.5516, found: 1348.5509.

6'-Allyl-1,3,2',2''',6'''-pentaazido-6,3',3'',5'',3''',4'''-hexa-O-benzyl-4',6'-O-benzylideneparomomycin (163): To a stirred solution of **162R** (15.0 mg, 0.011 mmol) under Ar in anhydrous acetonitrile (0.5 mL) was added benzaldehyde dimethyl acetal (5.2 mg, 0.033 mmol) followed by a catalytic amount of CSA (0.30 mg, 0.001 mmol) in one portion at room temperature. The resulting reaction mixture was stirred for 2 h at room temperature before it was quenched with TEA and concentrated under reduced pressure. The crude product was purified via silica gel chromatography eluting with 5% to 15% EtOAc in hexanes to give **163** (10.5 mg,

66%) as a yellow foam. $[\alpha]_D^{26}$= +65.0 (*c*=0.48, CH_2Cl_2); ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.61-6.98 (m, 30H, Ar-*H*), 6.21 (d, *J* = 3.8 Hz, 1H, H-1'), 6.03 (m, 1H, H-8'), 5.67 (d, *J* = 5.7 Hz, 1H, H-1''), 5.56 (s, 1H, Benzylidene), 5.18 (m, 1H, H-9'*CH*$_2$), 4.97 (d, *J* = 10.6 Hz, 1H, *CH*$_2$Ph), 4.94-4.87 (m, 2H, H-1''', *CH*$_2$Ph), 4.76 (d, *J* = 11.2 Hz, 1H, *CH*$_2$Ph), 4.73 (d, *J* = 10.6 Hz, 1H, *CH*$_2$Ph), 4.62 (d, *J* = 12.1 Hz, 1H, *CH*$_2$Ph), 4.57 (d, *J* = 11.8 Hz, 1H, *CH*$_2$Ph), 4.51 (d, *J* = 11.9 Hz, 1H, *CH*$_2$Ph), 4.49-4.39 (m, 3H, *CH*$_2$Ph), 4.35-4.2 (m, 3H, H-3'', H-4'', *CH*$_2$Ph), 4.25 (d, *J* = 12.1 Hz, 1H, *CH*$_2$Ph), 4.09 (t, *J* = 9.6 Hz, 1H, H-3'), 4.01-3.91 (m, 2H, H-2'', H-5), 3.82-3.75 (m, 3H, H-3''', H-4''', H-5''*CH*$_2$), 3.72 (d, *J* = 9.3 Hz, 1H, H-5'), 3.74-3.66 (m, 3H, H-6', H-5''', H-6'''*CH*$_2$), 3.65 (t, *J* = 9.1 Hz, 1H, H-4), 3.57 (dd, *J* = 10.5, 2.9 Hz, 1H, H-5''*CH*$_2$), 3.49-3.40 (m, 3H, H-1, H-3, H-4'), 3.35 (t, *J* = 2.4 Hz, 1H, H-4'''), 3.28 (t, *J* = 9.3 Hz, 1H, H-6), 3.12 (d, *J* = 2.4 Hz, 1H, H-2'''), 3.04 (dd, *J* = 10.1, 3.8 Hz, 1H, H-2'), 2.87 (dd, *J* = 13.0, 3.8 Hz, 1H, 6'''*CH*$_2$), 2.67 (m, 1H, H-7'*CH*$_2$), 2.42 (dt, *J* = 14.8, 7.4 Hz, 1H, H-7'*CH*$_2$), 2.23 (dt, *J* = 13.2, 4.6 Hz, 1H, H-2*CH*$_2$), 1.37 (q, *J* = 12.7 Hz, 1H, H-2*CH*$_2$); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 138.27, 138.06, 137.87, 137.59, 137.54, 136.98, 136.92, 134.30 (C-8'*CH*$_2$), 128.75, 128.66, 128.49, 128.40, 128.34, 128.31, 128.27, 128.18, 128.13, 127.80, 127.78, 127.72, 127.68, 127.50, 127.42, 127.23, 126.07 (arom.), 117.16 (C-9'*CH*$_2$), 106.13 (C-1''), 100.81 (C-Benzylidene), 98.60 (C-1'''), 96.06 (C-1'), 84.31 (C-6), 82.37 (C-3''), 82.11 (C-5), 81.79 (C-2''), 81.73 (C-4'), 78.92 (C-6'), 75.97 (C-3'), 75.47 (C-4''), 75.10 (C-*CH*$_2$Ph), 75.04 (C-4), 74.93 (C-5'''), 74.4 (C-*CH*$_2$Ph), 73.21 (C-*CH*$_2$Ph), 73.10 (C-*CH*$_2$Ph), 72.87 (C-3'''), 72.36 (C-*CH*$_2$Ph), 71.71 (C-*CH*$_2$Ph), 71.50 (C-2'''), 70.29 (C-5''), 67.05 (C-5'), 62.75 (C-2'), 60.35 (C-1), 59.99 (C-3), 57.24 (C-4'''), 51.11 (C-6'''), 35.98 (C-6'''), 32.55 (C-7'*CH*$_2$), 29.69 (C-2*CH*$_2$); ESI-HRMS: *m/z* calcd. for $C_{68}H_{74}N_{15}O_{14}BrNa$ $[M+Na]^+$ 1436.5829, found: 1436.5852.

4-*O*-(2'-Azido-3',6'-di-*O*-benzyl-9'-bromo-4',8'-anhydro-2',7',9'-trideoxy-D-erythro-*α*-D-gluco-nonapyranosyl)-5-O-[3''-O-(2''',6'''-diazido-3''',4'''-di-O-benzyl-2''',6'''-dideoxy-β-L-idopyranosyl)-2'',5''-di-O-benzyl-β-D-ribofuranosyl]-1,3-diazido-6-O-benzyl-2-deoxystreptamine (164): A stirred solution of **162*R*** (480.0 mg, 0.36 mmol) in anhydrous acetonitrile (5.0 mL) was treated with N-bromosuccinamide (66.4 mg, 0.37 mmol) at 0 ºC. The resulting reaction mixture was stirred at 0 ºC for 12 h. The resulted compound was extracted into ethyl acetate (10.0 mL) and was washed with brine (5.0 mL). The organic layer was concentrated to afford a yellow oil that was purified by chromatography on silica gel (EtOAc/Hexanes 3% to 30%) to afford **164** (196 mg, 38%), **165** (100 mg, 25%), and **166** (102 mg, 20%), as a white foam. $[\alpha]_D^{26}$= +73.0 (*c*=0.46, CH_2Cl_2); ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.54-7.11 (m, 30H, Ar-*H*), 6.17 (d, *J* = 3.7 Hz, 1H, H-1'), 5.74 (d, *J* = 5.7 Hz, 1H, H-1''), 5.07 (d, *J* = 11.1 Hz, 1H, *CH*$_2$Ph), 5.03 (d, *J* = 10.5 Hz, 1H, *CH*$_2$Ph), 4.95 (d, *J* = 1.9 Hz, 1H, H-1'''), 4.80 (d, *J* = 11.1 Hz, 1H, *CH*$_2$Ph), 4.75 (d, *J* = 10.6 Hz, 1H, *CH*$_2$Ph), 4.65 (d, *J* = 12.1 Hz, 1H, *CH*$_2$Ph), 4.63 (d, *J* = 11.8 Hz, 1H, *CH*$_2$Ph), 4.53- 4.49 (m, 3H, *CH*$_2$Ph), 4.43 (d, *J* = 12.0 Hz, 1H, *CH*$_2$Ph), 4.39 (m, 1H, H-4''), 4.37 (m, 1H, H-3''), 4.34 (m, 1H, *CH*$_2$Ph), 4.28 (d, *J* = 12.1 Hz, 1H, *CH*$_2$Ph), 4.14 (q, *J* = 2.7 Hz, 1H, H-6'), 4.06 (t, *J* = 5.2 Hz, 1H, H-2''), 4.04-3.95 (m, 3H, H-8', H-3', H-5), 3.88 (dd, *J* = 10.4, 2.2 Hz, 1H, H-5''), 3.86-3.81 (m, 2H, H-5', H-5'''), 3.80 (t, *J* = 2.9 Hz, 1H, H-3'''), 3.72 (dd, *J* = 13.0, 8.7 Hz, 1H, H-6'''*CH*$_2$), 3.66-3.56 (m, 3H, H-4', H-5'', H-4), 3.46-3.41 (m, 2H, H-1, H-3), 3.39 (m, 1H, H-2'''), 3.38-3.35 (m, 2H, H-9'*CH*$_2$), 3.32 (t, *J* = 9.4 Hz, 1H, H-6), 3.15 (t, *J* = 2.3 Hz, 1H, H-4'''), 2.96 (dd, *J* = 10.2, 3.8 Hz, 1H, H-2'), 2.88 (dd, *J* = 13.0, 3.6 Hz, 1H, H-6'''*CH*$_2$), 2.24 (dq, *J* = 13.1, 4.4 Hz, 1H, H-2*CH*$_2$), 2.02 (dt, *J* = 14.0, 2.8 Hz, 1H, H-7'*CH*$_2$), 1.62 (ddd, *J* = 14.2, 11.4, 2.7 Hz, 1H, H-7'*CH*$_2$), 1.41 (q, *J* = 12.7 Hz, 1H, H-2*CH*$_2$); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 138.37, 137.92, 137.00, 128.71, 128.55, 128.45, 128.38, 128.37,

128.32, 128.31, 128.30, 128.24, 127.86, 127.84, 127.77, 127.70, 127.64, 127.60, 127.53, 127.03 (arom.), 106.21 (C-1''), 98.68 (C-1'''), 96.50 (C-1'), 84.28 (C-6), 82.66 (C-2''), 82.30 (C-4''), 81.95 (C-5), 76.39 (C-3'), 75.65 (C-3''), 75.57 (C-4'), 75.06 (C-*CH*$_2$Ph), 74.93 (2C, C-4, C-*CH*$_2$Ph), 74.88 (C-*CH*$_2$Ph), 74.51 (C-5'''), 73.23 (C-3'''), 73.14 (C-*CH*$_2$Ph), 72.85 (C-*CH*$_2$Ph), 72.40 (C-*CH*$_2$Ph), 71.71 (C-8'), 71.49 (C-4'''), 70.35 (C-5''), 69.15 (C-5'), 65.08 (C-6'), 62.51 (C-2'), 60.28 (C-1), 60.05 (C-3), 57.29 (C-2'''), 51.20 (C-6'''*CH*$_2$), 35.81 (C-7'*CH*$_2$), 34.91 (C-9'*CH*$_2$), 32.45 (C-2*CH*$_2$); ESI-HRMS: *m/z* calcd. for $C_{68}H_{74}N_{15}O_{14}BrNa$ $[M+Na]^+$ 1428.4600, found: 1428.4596.

4-O-(2'-Azido-3'-O-benzyl-9'-bromo-5',8'-anhydro-2',7',9'-trideoxy-D-erythro-α-D-gluconononafuranosyl)-5-O-[3''-O-(2''',6'''-diazido-2''',6'''-dideoxy-3''',4'''-di-O-benzyl-β-L-idopyranosyl)-2'',5''-di-O-benzyl-β-D-ribofuranosyl]-1,3-diazido-6-O-benzyl-2-deoxystreptamine (165): $[\alpha]_D^{26}$= +60.2 (*c*=0.42, Dichloromethane); ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.48-6.97 (m, 30H, arom.), 5.91 (d, *J* = 4.6 Hz, 1H, H-1'), 5.32 (d, *J* = 3.7 Hz, 1H, H-1''), 4.83 (d, *J* = 1.9 Hz, 1H, H-1'''), 4.81 (s, 2H, *CH*$_2$Ph), 4.63 (d, *J* = 12.1 Hz, 1H, *CH*$_2$Ph), 4.59 (d, *J* = 11.7 Hz, 1H, *CH*$_2$Ph), 4.54 (d, *J* = 11.7 Hz, 1H, *CH*$_2$Ph), 4.48 (d, *J* = 11.9 Hz, 1H, *CH*$_2$Ph), 4.42 (m, 1H, *CH*$_2$Ph), 4.40 (m, 1H, *CH*$_2$Ph), 4.38 (m, 3H, *CH*$_2$Ph, H-6'), 4.36-4.32 (m, 2H, *CH*$_2$Ph, H-3''), 4.30-4.23 (m, 3H, H-4' H-4'', H-8'), 4.17 (d, *J* = 11.7 Hz, 1H, *CH*$_2$Ph), 4.12-4.09 (m, 1H, H-3'), 4.09-4.07 (m, 1H, H-2'), 4.00 (dd, *J* = 8.9, 3.5 Hz, 1H, H-5'), 3.86-3.80 (m, 2H, H-2'', H-5'''), 3.77 (t, *J* = 3.0 Hz, 1H, H-3'''), 3.73 (dd, *J* = 10.8, 2.2 Hz, 1H, H-5''*CH*$_2$Ph), 3.67-3.64 (m, 1H, H-6'''*CH*$_2$), 3.64-3.59 (m, 1H, H-5), 3.58 (dd, *J* = 10.8, 4.4 Hz, 1H, H-5''*CH*$_2$Ph), 3.52-3.44 (m, 3H, H-4, H-9'*CH*$_2$), 3.41 (m, 1H, H-2'''), 3.40-3.32 (m, 2H, H-1, H-3), 3.20 (t, *J* = 9.5 Hz, 1H, H-6), 3.17 (d, *J* = 2.4 Hz, 1H, H-4'''), 2.95 (dd, *J* = 13.0, 4.0 Hz, 1H, H-6'''*CH*$_2$), 2.88 (s, 1H, OH), 2.31-2.24 (m, 1H, H-7' *CH*$_2$), 2.21 (dt, *J* = 13.3, 4.5 Hz, 1H, H-

2*CH₂*), 2.05 (m, 1H, H-7'*CH₂*), 1.35 (q, *J* = 12.8 Hz, 1H, H-2 *CH₂*); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 128.67, 128.52, 128.43, 128.35, 128.33, 128.29, 128.24, 128.22, 127.85, 127.70, 127.66, 127.54, 127.47 (Ar-*C*.), 107.76 (C-1''), 102.97 (C-1), 98.27 (C-1'''), 83.54 (C-5), 83.48 (C-6), 82.10 (C-3'), 81.65 (C-2''), 81.04 (C-4''), 80.37 (C-5'), 79.24 (C-8'), 79.12 (C-4), 78.14 (C-4'), 75.33 (C-*CH₂Ph*), 74.70 (C-3''), 74.35 (C-5'''), 72.96 (C-3'''), 72.91 (C-*CH₂Ph*), 72.86 (C-6'), 72.71(C-*CH₂Ph*), 72.50 (C-*CH₂Ph*), 72.47 (C-*CH₂Ph*), 71.79 (C-*CH₂Ph*), 71.55 (C-4'''), 70.14 (C-5''*CH₂*), 65.76 (C-2'), 60.47 (C-1), 59.67 (C-3), 57.48 (C-2'''), 51.13 (C-6'''*CH₂*), 37.75 (C-7'*CH₂*), 35.73 (C-9'*CH₂*), 32.42 (C-2*CH₂*); ESI-HRMS: *m/z* calcd. for $C_{68}H_{74}N_{15}O_{14}BrNa$ $[M+Na]^+$ 1428.4600, found: 1428.4559.

4-O-(2'-Azido-3'-O-benzyl-9'-bromo-5',8'-anhydro-2',7',9'-trideoxy-L-threo-α-D-gluco-nonafuranosyl)-5-O-[3''-O-(2''',6'''-diazido-2''',6'''-dideoxy-3''',4'''-di-O-benzyl-β-L-idopyranosyl)-2'',5''-di-O-benzyl-β-D-ribofuranosyl]-1,3-diazido-6-O-benzyl-2-deoxystreptamine (166): $[\alpha]_D^{26}$= +63.7 (*c*=0.40, Dichloromethane); ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.56-7.06 (m, 30H, arom.), 5.98 (d, *J* = 4.5 Hz, 1H, H-1'), 5.44 (d, *J* = 4.3 Hz, 1H, H-1''), 4.93-4.86 (m, 2H, H-1''', *CH₂*Ph), 4.82 (d, *J* = 10.8 Hz, 1H, *CH₂*Ph), 4.66 (d, *J* = 12.0 Hz, 1H, *CH₂*Ph), 4.61-4.55 (m, 2H, *CH₂*Ph), 4.51 (m, 2H, *CH₂*Ph), 4.47-4.43 (m, 2H, *CH₂*Ph), 4.42-4.34 (m, 4H, H-4', H-3'', *CH₂*Ph, H-8'), 4.34-4.27 (m, 5H, H-6', H-4'', *CH₂*Ph), 4.19 (t, *J* = 4.4 Hz, 1H, H-3'), 4.01 (dd, *J* = 6.1, 3.3 Hz, 1H, H-5'), 3.92-3.88 (m, 2H, H-2'', H-2'), 3.86 (m, 1H, H-5'''), 3.81 (m, 2H, H-3''', H-5''*CH₂*), 3.74-3.66 (m, 2H, H-4, H-6'''*CH₂*), 3.64 (dd, *J* = 7.4, 3.4 Hz, 1H, H-5''*CH₂*), 3.62 (m, 1H, H-5), 3.45 (m, 2H, H-2''', H-9'*CH₂*), 3.43-3.36 (m, 2H, H-3, H-9'*CH₂*), 3.31 (ddd, *J* = 12.7, 9.7, 4.5 Hz, 1H, H-1), 3.25 (t, *J* = 9.5 Hz, 1H, H-6), 3.19 (d, *J* = 2.3 Hz, 1H, H-4'''), 2.96 (dd, *J* = 13.0, 3.9 Hz, 1H, H-6'''*CH₂*), 2.18 (dt, *J* = 13.3, 4.6 Hz, 1H, H-2*CH₂*), 2.04 (ddd, *J* = 13.2, 6.3, 3.2 Hz, 1H, H-7'*CH₂*), 1.93 (m, 1H, H-7'*CH₂*), 1.36 (q, *J* = 12.8

Hz, 1H, H-2CH_2); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 128.72, 128.57, 128.47, 128.39, 128.38, 128.36, 128.35, 128.26, 128.09, 127.99, 127.89, 127.77, 127.75, 127.66, 127.63, 127.56, 127.30 (Ar-*C*), 107.35 (C-1''), 102.59 (C-1'), 98.43 (C-1'''), 85.03 (C-5'), 83.68 (C-6), 83.24 (C-4'''), 81.89 (C-6'), 81.72 (C-3'), 79.29 (C-2''), 78.56 (C-4), 77.64 (C-5), 75.33 (C-CH_2Ph), 74.97 (C-8'), 74.42 (C-3''), 73.12 (C-5'''), 73.02 (C-CH_2Ph), 72.89 (C-CH_2Ph), 72.71(C-CH_2Ph), 72.50 (C-CH_2Ph), 71.80 (C-CH_2Ph), 71.55 (C-3'''), 70.26 (C-5''CH_2), 66.16 (C-2'), 60.52 (C-3), 59.44 (C-1), 57.48 (C-2'''), 51.17 (C-6'''CH_2), 38.76 (C-7'), 35.29 (C-9'CH_2), 32.35 (C-2CH_2); ESI-HRMS: *m/z* calcd. for $C_{68}H_{74}N_{15}O_{14}BrNa$ $[M+Na]^+$ 1428.4600, found: 1428.4602.

4-*O*-(2'-Azido-3',6'-di-*O*-benzyl-9'-bromo-4',8'-anhydro-2',7',9'-trideoxy-D-threo-α-D-gluco-nonapyranosyl)-5-O-[3''-O-(2''',6'''-diazido-3''',4'''-di-O-benzyl-2''',6'''-dideoxy-β-L-idopyranosyl)-2'',5''-di-O-benzyl-β-D-ribofuranosyl]-1,3-diazido-6-O-benzyl-2-deoxystreptamine (170): A solution of **164** (150.0 mg, 0.11 mmol) in dry dichloromethane (5.0 mL) was treated with Dess–Martin periodinane (90.6 mg, 0.21 mmol) and Sodium bicarbonate (18.0 mg, 0.21 mmol), stirred for 9 h under Ar at room temperature. The reaction mixture was washed with water followed by brine, dried, and concentrated under reduced pressure. The crude mixture (ketone) (115 mg, 0.08 mmol) was stirred with $NaBH_4$ (6.2 mg, 0.16 mmol) in methanol (4.0 mL) for 30 min. The reaction mixture was neutralized with acetic acid and concentrated under reduced pressure. The crude diastereomer (3:1 ratio) was separated by silica gel column using 30% EtOAc in hexanes to give the title compound **170** (66 mg, 58%) as a white foam. $[\alpha]_D^{26}$= +67.9 (*c*=0.19, CH_2Cl_2); ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.56-6.98 (m, 30H), 6.13 (d, *J* = 3.9 Hz, 1H, H-1'), 5.65 (d, *J* = 5.2 Hz, 1H, H-1''), 5.02 (d, *J* = 11.0 Hz, 1H, CH_2Ph), 4.96 (d, *J* = 10.7 Hz, 1H, CH_2Ph), 4.88 (d, *J* = 1.9 Hz, 1H, H-1'''), 4.75 (m, 2H, CH_2Ph), 4.63 (d, *J* = 12.1 Hz, 1H, CH_2Ph), 4.55 (d, *J* = 11.7 Hz, 1H, CH_2Ph), 4.52 (d, *J* = 11.9 Hz, 1H, CH_2Ph), 4.46

(d, *J* = 11.9 Hz, 1H, *CH*$_2$Ph), 4.42 (m, 2H, *CH*$_2$Ph), 4.36-4.31 (m, 3H, H-3'', H-4'', *CH*$_2$Ph), 4.26 (d, *J* = 12.1 Hz, 1H, *CH*$_2$Ph), 4.01-3.97 (m, 2H, H-2'', H-3'), 3.96- 3.91 (m, 1H, H-5'), 3.84-3.75 (m, 3H, H-5''', H-3''', H-5''*CH*$_2$), 3.72-3.62 (m,5H, H-4', H-4, H- 6'''*CH*$_2$, H-6', H-8'), 3.59 (dd, *J* = 10.5, 2.9 Hz, 1H, H-5''*CH*$_2$), 3.52-3.35 (m, 5H, H-1, H-3, H-9'*CH*$_2$, H-2'''), 3.31 (t, *J* = 9.2 Hz, 1H, H-5), 3.13 (t, *J* = 2.3 Hz, 1H, H-4'''), 3.09-3.02 (m, 2H, H-2', H-6), 2.89 (dd, *J* = 13.0, 3.8 Hz, 1H, H-6'''*CH*$_2$), 2.33-2.15 (m, 2H, H-7'*CH*$_2$, H-2*CH*$_2$), 1.53-1.38 (m, 2H, H-7'*CH*$_2$, H-2*CH*$_2$); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 128.68, 128.52, 128.43, 128.34, 128.31, 128.30, 128.28, 128.21, 127.84, 127.79, 127.72, 127.68, 127.46, 127.28 (Ar-*C*), 106.35 (C-1''), 98.55 (C-1'''), 95.83 (C-1'), 83.94 (C-5), 82.25 (C-2''), 81.98 (C-5'), 79.94 (C-6), 76.41 (C-3'), 75.52 (C-3''), 75.37 (C-4, C-4''), 74.95 (2C, C-*CH*$_2$Ph), 72.59 (C-6'), 74.41 (2C, C-5''',C-8'), 73.17 (C-*CH*$_2$Ph), 73.07 (C-*CH*$_2$Ph), 72.86 (C-3'''), 72.41 (C-*CH*$_2$Ph), 71.72 (C-*CH*$_2$Ph), 71.49 (C-4'''), 70.30 (C-5''*CH*$_2$), 69.67 (C-4'), 62.66 (C-2'), 60.23 (C-1), 59.98 (C-3), 57.30 (C-2'''), 51.13 (C-6'''*CH*$_2$), 37.21 (C-7'*CH*$_2$), 34.07 (C-9'*CH*$_2$), 32.31 (C-2*CH*$_2$); ESI-HRMS: *m/z* calcd. for $C_{68}H_{74}N_{15}O_{14}BrNa$ $[M+Na]^+$ 1428.4600, found: 1428.4626.

4-*O*-(2',6'-Diazido-3',6'-di-*O*-benzyl-9'-bromo-4',8'-anhydro-2',6',7',9'-tetradeoxy-D-threo-α-D-gluco-nonapyranosyl)-5-O-[3''-O-(2''',6'''-diazido-3''',4'''-di-O-benzyl-2''',6'''-dideoxy-β-L-idopyranosyl)-2'',5''-di-O-benzyl-β-D-ribofuranosyl]-1,3-diazido-6-O-benzyl-2-deoxystreptamine (169): To a stirred solution of **164** (90.0 mg, 0.06 mmol) in dry dichloromethane (1.0 mL) at room temperature was added dry pyridine (21.7 mg, 0.27 mmol) in one portion. Triflic anhydride (40 mg, 0.14 mmol) was added to the reaction mixture at 0 ºC under Ar. The reaction mixture was stirred at 0 ºC for 1 h and was quenched with sat. $NaHCO_3$ solution. The reaction mixture was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was stirred with sodium azide (20.0 mg,

0.30 mmol) in dry DMF (0.5 mL) at room temperature. The resulting reaction mixture was stirred at room temperature for 6 h after which the solvent was evaporated under reduced pressure. The residue was dissolved in dichloromethane (2 mL) and washed with water, brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified via silica gel chromatography (eluent: 2% to 20% EtOAc in hexanes) to give **169** (45.0 mg, 49%) as a colorless oil. $[\alpha]_D^{26}$= +51.0 (*c*=0.6, CH_2Cl_2); ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.65-6.99 (m, 30H, Ar-*H*), 6.23 (d, *J* = 3.8 Hz, 1H, H-1'), 5.69 (d, *J* = 5.8 Hz, 1H, H-1''), 4.97 (d, *J* = 11.1 Hz, 1H, *CH*$_2$Ph), 4.95 (m, 2H, *CH*$_2$Ph, H-1'''), 4.74 (d, *J* = 11.0 Hz, 1H, *CH*$_2$Ph), 4.71 (d, *J* = 10.7 Hz, 1H, *CH*$_2$Ph), 4.63 (d, *J* = 7.4 Hz, 1H, *CH*$_2$Ph), 4.61 (d, *J* = 7.1 Hz, 1H, *CH*$_2$Ph), 4.49 (m, 2H, *CH*$_2$Ph), 4.46 (d, *J* = 9.1 Hz, 1H, *CH*$_2$Ph), 4.42 (d, *J* = 11.5 Hz, 1H, *CH*$_2$Ph), 4.32 (d, *J* = 12.0 Hz, 1H, *CH*$_2$Ph), 4.28 (m, 2H, H-3'', H-4'), 4.24 (d, *J* = 12.1 Hz, 1H, *CH*$_2$Ph), 4.00-3.91 (m, 3H, H-2'', H-3', H-5), 3.83-3.77 (m, 3H, H-3''', H-4'', H-5''*CH*$_2$), 3.76 (t, *J* = 2.9 Hz, 1H, H-5'''), 3.70 (t, *J* = 9.54 Hz, 1H, H-6), 3.67 (m, 1H, H-6'''*CH*$_2$), 3.63 (m, 1H, H-8'), 3.56 (dd, *J* = 10.4, 3.0 Hz, 1H, H-5''*CH*$_2$), 3.46 (m, 3H, H-1, H-3, H-5'), 3.40-3.32 (m, 3H, H-2''', H-9'*CH*$_2$), 3.31 (t, *J* = 9.54 Hz 1H, H-6), 3.11 (t, *J* = 2.3 Hz, 1H, H-4'''), 3.03-2.94 (m, 2H, H-2', H-6'), 2.84 (dd, *J* = 13.0, 3.8 Hz, 1H, H-6'''*CH*$_2$), 2.25 (dt, *J* = 13.2, 4.6 Hz, 1H, H-2*CH*$_2$), 2.15 (m, 1H, H-7'*CH*$_2$), 1.44 (q, *J* = 12.84 Hz 1H, H-2*CH*$_2$), 1.41 (q, *J* = 12.10 Hz, 1H, H-7'*CH*$_2$); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 138.32, 138.09, 137.92, 137.64, 136.99, 136.93, 128.67, 128.49, 128.42, 128.34, 128.32, 128.29, 128.25, 128.18, 127.81, 127.74, 127.70, 127.69, 127.43, 127.33, 127.17 (Ar-*C*), 106.09 (C-1''), 98.57 (C-1'''), 95.64 (C-1'), 84.39 (C-6), 82.62 (C-2''), 82.11 (C-3''), 81.59 (C-5), 80.65 (C-6'), 76.21 (C-3'), 75.55 (C-4'), 75.47 (C-8'), 75.04 (C-*CH*$_2$Ph, C-4), 75.00 (C-*CH*$_2$Ph), 74.45 (C-4''), 73.33 (C-*CH*$_2$Ph), 73.16 (C-*CH*$_2$Ph), 72.80 (C-5'''), 72.33 (C-*CH*$_2$Ph), 71.66 (C-*CH*$_2$Ph), 71.41 (2C, C-4''', C-3'''), 70.32 (C-5''), 62.53 (C-2'), 60.36 (C-5'), 59.93 (C-1),

59.81 (C-3), 57.23 (C-2'''), 51.08 (C-6'''), 35.09 (C-7'), 33.48 (C-9'), 32.46 (C-2); ESI-HRMS: *m/z* calcd. for $C_{68}H_{73}N_{18}O_{13}BrNa$ $[M+Na]^+$ 1451.4686, found: 1451.4667.

4-*O*-(2',6'-Diazido-3',6'-di-*O*-benzyl-9'-bromo-4',8'-anhydro-2',6',7',9'-tetradeoxy-D-erythro-α-D-gluco-nonapyranosyl)-5-O-[3''-O-(2''',6'''-diazido-3''',4'''-di-O-benzyl-2''',6'''-dideoxy-β-L-idopyranosyl)-2'',5''-di-O-benzyl-β-D-ribofuranosyl]-1,3-diazido-6-O-benzyl-2-deoxystreptamine (172): To a stirred solution of **170** (70.0 mg, 0.05 mmol) in dry dichloromethane (1.5 mL) at room temperature was added dry pyridine (17.0 mg, 0.22 mmol) in one portion. Triflic anhydride (31.0 mg, 0.11 mmol) was added to the reaction mixture at 0 ºC under Ar. The reaction mixture was stirred at 0 ºC for 1 h and was quenched with sat. $NaHCO_3$ solution and washed with brine, dried, filtered, and concentrated under reduced pressure. The crude product was stirred with sodium azide (32.0 mg, 0.49 mmol) in dry DMF (0.7 mL) at room temperature. The resulting reaction mixture was stirred at room temperature for 4 h after which the solvent was evaporated under reduced pressure. The residue was dissolved in dichloromethane (3.0 mL) and washed with water, brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified via silica gel chromatography eluting with 2% to 30% EtOAc in hexanes to give **172** (61.0 mg, 67%) as an off-white foam. $[\alpha]_D^{26}$= +132.1 (*c*=0.11, Dichloromethane); ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.43-7.38 (m, 30H, Ar-*C*), 6.06 (d, *J* = 3.8 Hz, 1H, H-1'), 5.62 (d, *J* = 5.5 Hz, 1H, H-1''), 4.97 (d, *J* = 11.2 Hz, 1H, *CH*$_2$Ph), 4.94 (d, *J* = 10.7 Hz, 1H, *CH*$_2$Ph), 4.84 (d, *J* = 1.9 Hz, 1H, H-1'''), 4.74 (d, *J* = 11.2 Hz, 1H, *CH*$_2$Ph), 4.70 (d, *J* = 10.7 Hz, 1H, *CH*$_2$Ph), 4.60 (d, *J* = 12.0 Hz, 1H, *CH*$_2$Ph), 4.57-4.51 (m, 2H, *CH*$_2$Ph), 4.42 (d, 2H, *CH*$_2$Ph), 4.39 (d, *J* = 11.9 Hz, 1H, *CH*$_2$Ph), 4.30 (d, *J* = 11.9 Hz, 1H, *CH*$_2$Ph), 4.27-4.22 (m, 3H, H-3'', H-4'', *CH*$_2$Ph), 4.11 (dd, *J* = 5.7, 2.2 Hz, 1H, H-6'), 4.02 (dd, *J* = 10.0, 3.3 Hz, 1H, H-5'), 3.95-3.89 (m, 3H, H-2'', H-5, H-3'), 3.86 (m, 1H,

H-8'), 3.80-3.71 (m, 3H, H-5"CH_2, H-5"', H-3"'), 3.68-3.57 (m, 3H, H-4', H-4, H-6"'CH_2), 3.53 (dd, J = 10.5, 3.5 Hz, 1H, H-5"CH_2), 3.41 (m, 2H, H-1, H-3), 3.36-3.31 (m, 3H, H-2"', H-9'CH_2), 3.29 (t, J = 9.2 Hz, 1H, H-6), 3.10 (d, J = 2.4 Hz, 1H, H-4"'), 3.04 (dd, J = 10.2, 3.8 Hz, 1H, H-2'), 2.88 (dd, J = 12.9, 4.1 Hz, 1H, H-6"'CH_2), 2.24 (dt, J = 13.3, 4.6 Hz, 1H, H-2CH_2), 1.98 (dt, J = 14.0, 2.7 Hz, 1H, H-7'CH_2), 1.69 (ddd, J = 14.2, 11.3, 3.2 Hz, 1H, H-7'CH_2), 1.41 (q, J = 12.7 Hz, 1H, H-2CH_2); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 138.31, 138.20, 137.79, 137.64, 137.01, 136.94, 128.67, 128.49, 128.40, 128.34, 128.32, 128.30, 128.27, 128.18, 127.83, 127.80, 127.77, 127.59, 127.56, 127.54 (Ar-*C*), 106.02 (C-1"), 98.58 (C-1"'), 96.49 (C-1'), 83.97 (C-6), 82.34 (C-4"), 82.08 (C-5), 81.39 (C-2"), 76.67 (C-3'), 76.38 (C-4'), 75.51 (C-4), 75.42 (C-3"), 75.05 (C-CH_2Ph), 74.85 (C-CH_2Ph), 74.27 (C-5"'), 73.31 (C-CH_2Ph), 73.23 (C-CH_2Ph), 72.88 (C-3"'), 72.38 (C-CH_2Ph), 71.72 (C-CH_2Ph), 71.47 (C-8'), 71.41 (C-4"'), 70.05 (C-5"CH_2), 69.26 (C-5'), 62.28 (C-2'), 60.21 (C-1), 59.89 (C-3), 57.63 (C-6'), 57.30 (C-2"'), 51.03 (C-6"'CH_2), 34.86 (C-7'CH_2), 34.27 (C-9'CH_2), 32.41 (C-2CH_2); ESI-HRMS: *m/z* calcd. for $C_{68}H_{73}N_{18}O_{13}BrNa$ $[M+Na]^+$ 1451.4686, found: 1451.4679.

General procedure A for hydrogenolysis. To a stirred solution of substrate (0.02 mmol) in a mixture of *p*-dioxane (0.5 mL), deionized water H_2O (0.2 mL), and glacial AcOH (20 µL) was treated with Pd/C on carbon (20 wt. %, 100 % loading) and stirred for 48h at room temperature under 40 psi of hydrogen. After completion, the reaction mixture was filtered through Celite® and the filtrate was evaporated under reduced pressure to give crude product. The residue was dissolved in 0.002 M aqueous AcOH (2.0 mL) and then charged to a Sephadex column (CM Sephadex C-25, 5.0 g). The Sephadex column was eluted with deionized water H_2O (50 mL), 0.5% aqueous NH_4OH (40 mL), and 1.5% NH_4OH (40 mL). The product-containing fractions were combined and evaporated to give the product in the form of the free base, which

was taken up in H_2O (2 mL) and treated with glacial acetic acid (10 eq). The resulting solution was lyophilized to give the product in the form of the acetate salt.

4-O-(2'-Amino-4',8'-anhydro-2',7',9'-trideoxy-D-erythro-α-D-gluco-nonapyranosyl)-5-O-[3-O-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-2-deoxystreptamine.5AcOH (154): Following general procedure A, Compound **154** (12.7 mg, 63%) was synthesized from **164**, as a white foam. $[\alpha]_D^{26}$= +25.7 (*c*=0.37, H_2O);^{1}H NMR (600 MHz, D_2O) δ 5.70 (d, *J* = 4.1 Hz, 1H, H-1'), 5.25 (d, *J* = 2.2 Hz, 1H, H-1''), 5.14 (d, *J* = 1.8 Hz, 1H, H-1'''), 4.38 (dd, *J* = 7.0, 4.8 Hz, 1H, H-3''), 4.26 (dd, *J* = 4.9, 2.1 Hz, 1H, H-2''), 4.16 (dd, *J* = 6.9, 4.0 Hz, 1H, H-5'''), 4.07 (m, 3H, H-6', H-3''', H-4''), 3.87-3.80 (m, 3H, H-3', H-8', H-4), 3.77 (dd, *J* = 12.5, 2.9 Hz, 1H, H-5''*CH₂*), 3.71 (t, *J* = 8.1 Hz, 1H, H-6), 3.67 (br s, 1H, H-4'''), 3.62 (dd, *J* = 12.4, 4.6 Hz, 1H, H-5''*CH₂*), 3.58 (dd, *J* = 10.0, 2.8 Hz, 1H, H-5'), 3.54-3.45 (m, 2H, H-4', H-5), 3.43 (br s, 1H, H-2'''), 3.28 (dd, *J* = 13.7, 6.8 Hz, 1H, H-6'''*CH₂*), 3.25-3.10 (m, 3H, H- H-6'''*CH₂*, H-1, H-3), 2.22 (dt, *J* = 12.8, 4.3 Hz, 1H, H-2*CH₂*), 1.76 (m, 1H, H-7'*CH₂*), 1.62-1.37 (m, 2H, H-2*CH₂*, H-7'*CH₂*), 1.05 (d, *J* = 6.2 Hz, 3H, H-9'*CH₃*); ^{13}C NMR (151 MHz, Deuterium Oxide) δ 181.13 (C-CH_3*CO*OH), 110.01 (C-1''), 96.30 (C-1'), 95.28 (C-1'''), 84.84 (C-6), 81.10 (C-3''), 77.93 (C-8'), 74.93 (C-3'''), 73.45 (C-2''), 73.37 (C-5), 72.80 (C-4''), 70.29 (C-5'''), 70.21 (C-5'), 69.11 (H-4'), 67.62 (C-3'''), 67.30 (C-3'), 67.22 (C-4'''), 64.37 (C-6'), 59.85 (C-5''*CH₂*), 54.39 (C-2'), 50.81 (C-2'''), 50.03 (C-1), 48.64 (C-3), 40.34 (C-6'''*CH₂*), 38.86 (C-7'*CH₂*), 29.60 (C-2*CH₂*), 23.11 (C-*CH₃*COOH), 19.81 (C-9'*CH₃*); ESI-HRMS: *m/z* calcd. for $C_{26}H_{49}N_5O_{14}Na$ $[M+Na]^+$ 678.3174, found: 678.3166.

4-O-(2'-Amino-4',8'-anhydro-2',7',9'-trideoxy-D-threo-α-D-gluco-nonapyranosyl)-5-O-[3-O-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-2-deoxystreptamine.5AcOH (155): Following general procedure A, Compound 155 (7.0 mg,

40%) was synthesized from **170**, as a white foam. [α]D26= +30.5 (c=0.20, H2O); 1H NMR (600 MHz, D2O) δ 5.53 (d, J = 4.1 Hz, 1H, H-1'), 5.20 (d, J = 2.6 Hz, 1H, H-1"), 5.11 (s, 1H, H-1'"), 4.34 (t, J = 5.1 Hz, 1H, H-3"), 4.19 (br s, 1H, H-2"), 4.13 (t, J = 5.3 Hz, 1H, H-5'"), 4.03 (m, 2H, H-3'", H-4"), 3.79 (t, J = 9.9 Hz, 1H, H-3'), 3.77-3.68 (m, 4H, H-4, H-6, H-6', 5"CH2), 3.64 (d, J = 3.2 Hz, 1H, H-4'"), 3.62-3.54 (m, 2H, H-8', 5"CH2), 3.50 (t, J = 9.7 Hz, 1H, H-5), 3.40 (s, 1H, H-2'"), 3.37 (t, J = 9.4 Hz, 1H, H-5'), 3.31-3.22 (m, 3H, H-3, H-2', 6'"CH2), 3.21-3.15 (m, 2H, H-1, 6'"CH2), 3.12 (t, J = 9.5 Hz, 1H, H-4'), 2.25 (d, J = 11.8 Hz, 1H, H-2CH2), 1.96 (dd, J = 12.1, 3.3 Hz, 1H, H-7'CH2), 1.66-1.52 (m, 1H, H-2CH2), 1.25 (q, J = 11.9 Hz, 1H, H-7'CH2), 1.06 (d, J = 6.2 Hz, 3H, 9'-CH3). 13C NMR (151 MHz, D2O) δ 180.92 (C-CH3COOH), 109.70 (C-1"), 96.65 (C-1'), 95.34 (C-1'"), 84.05 (C-6), 81.21 (C-4"), 79.17 (C-4), 77.20 (C-4'), 75.00 (C-3"), 73.50 (C-5'), 73.29 (C-8'), 73.00 (C-2"), 72.47 (C-5), 70.11 (C-5'"), 68.30 (C-6'), 67.56 (C-4'"), 67.18 (2C-3', 3'"), 60.02 (C-5"CH2), 54.34 (C-2'), 50.74 (C-2'"), 49.69 (C-3), 48.91 (C-1), 40.30 (C-6'"CH2), 40.16 (C-7'CH2), 28.91 (C-2), 22.94 (C-CH3COOH), 19.80 (C-9'CH3); ESI-HRMS: m/z calcd. for C26H50N5O14 [M+H]+ 656.3354, found: 656.3371.

4-O-(2',6'-Diamino-4',8'-anhydro-2',6',7',9'-tetradeoxy-D-threo-α-D-gluco-nonapyranosyl)-5-O-(β-paramobiosyl)-2-deoxystreptamine.6AcOH (157): Following general procedure A, Compound **157** (6.2 mg, 40%) was obtained from **169**, as a white foam. $[\alpha]_D^{26}$= +33.8 (*c*=0.13, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 5.87 (d, *J* = 4.0 Hz, 1H, H-1'), 5.25 (d, *J* = 2.6 Hz, 1H, H-1"), 5.11 (s, 1H, H-1'"), 4.31 (t, *J* = 5.7 Hz, 1H, H-3"), 4.20 (dd, *J* = 5.0, 2.6 Hz, 1H, H-2"), 4.12 (d, *J* = 5.5 Hz, 1H, H-5'"), 4.04 (br s, 2H, H-3'", H-4"), 3.90 (t, *J* = 10.1 Hz, 1H, H-3'), 3.73 (m, 3H, H-6, H-4, H-5"*CH₂*), 3.64 (br s, 1H, H-8'), 3.61-3.53 (m, 2H, H-5', H-5"*CH₂*), 3.47 (t, *J* = 8.8 Hz, 1H, H-5), 3.40 (m, 2H, H-2'", H-6'), 3.27 (dd, *J* = 11.0, 4.0 Hz, 1H, H-2'), 3.25-3.21 (m, 1H, H-6'"*CH₂*), 3.21-3.09 (m, 5H, H-4'", H-1, H-3, H-4', H-6'"*CH₂*), 2.19 (dt, *J* =

12.6, 4.3 Hz, 1H, H-2CH_2), 2.10-2.00 (m, 1H, H-7'CH_2), 1.74 (s, 18H, CH_3COOH), 1.57 (q, *J* = 12.6 Hz 1H, H-2CH_2), 1.42 (q, *J* = 12.2 Hz, 1H, H-7'CH_2), 1.08 (q, *J* = 6.1 Hz 3H, H-9'CH_2). ^{13}C NMR (151 MHz, D_2O) δ 180.81 (C-CH_3COOH), 109.97 (C-1''), 95.40 (2C-1',1''), 84.92 (C-6), 81.44 (C-4''), 77.99 (C-4'), 76.72 (C-3''), 74.96 (C-4), 73.47 (C-2''), 72.92 (C-4'), 72.74 (C-5), 70.06 (C-5'''), 69.91 (C-5'), 67.53 (C-3'''), 67.20 (C-8'), 65.95 (C-3'), 60.02 (C-5''), 54.11 (C-2'), 50.72 (C-2'''), 50.36 (C-6'), 49.94 (C-1), 48.44 (C-3), 40.29 (C-6'''CH_2), 35.83 (C-7'CH_2), 26.36 (C-2CH_2) 22.87 (C-CH_3COOH), 19.60 (C-9'CH_3); ESI-HRMS: *m/z* calcd. for $C_{26}H_{51}N_6O_{13}$ $[M+H]^+$ 655.3514, found: 655.3505.

4-O-(2',6'-Diamino-4',8'-anhydro-2',6',7',9'-tetradeoxy-D-erythro-α-D-gluco-nonapyranosyl)-5-O-(β-paramobiosyl)-2-deoxystreptamine.6AcOH (156): To a Compound **172** (20.0 mg, 0.014 mmol) in a mixture of *p*-dioxane (0.5 mL), deionized water H_2O (0.2 mL), and 0.1 N NaOH (0.1 mL) was treated with Pd/C on carbon (20 mg, 20 wt. %) and stirred for 8h at room temperature under 40 psi of hydrogen. Added 10% AcOH (0.2 mL) and stirred for 20h at room temperature under 40 psi of hydrogen. After completion, the reaction mixture was filtered through Celite®, evaporated under reduced pressure, and the residue was dissolved in AcOH (1 mL) and then charged to a Sephadex column. The Sephadex column was eluted with deionized water H_2O (50 mL), 0.5% aqueous NH_4OH (40 mL), and 1.5% NH_4OH (40 mL) to give the **156** (8.5 mg, 60%) as a white form. $[\alpha]_D^{26}$= +41.8 (*c*=0.17, H_2O); ^{1}H NMR (600 MHz, D_2O) δ 5.74 (d, *J* = 4.2 Hz, 1H, H-1'), 5.24 (d, *J* = 2.7 Hz, 1H, H-1''), 5.13 (d, *J* = 1.8 Hz, 1H, H-1'''), 4.33 (dd, *J* = 6.4, 5.0 Hz, 1H, H-3''), 4.21 (dd, *J* = 5.0, 2.7 Hz, 1H, H-2''), 4.14 (td, *J* = 4.4, 2.0 Hz, 1H, H-5'''), 4.06 (br s, 2H, H-4'', H-3'''), 3.91-3.83 (m, 2H, H-3', H-5'), 3.81-3.70 (m, 5H, H-6', H-6, H-4, H-8', 5''CH_2), 3.66 (br s, 1H, H-4'''), 3.59 (dd, *J* = 12.4, 4.8 Hz, 1H, H-5''CH_2), 3.50 (t, *J* = 9.2 Hz, 1H, H-5), 3.42 (br s, 1H, H-2'''), 3.39 (t, *J* = 9.4 Hz, 1H, H-4') 3.32-3.18 (m, 4H, H-2', H-

1, H-6'''CH_2), 3.18-3.11 (m, 1H, H-3), 2.25 (dt, *J* = 12.8, 4.4 Hz, 1H, H-2CH_2), 1.92 (dt, *J* = 15.8, 2.4 Hz, 1H, H-7'CH_2), 1.82-1.76 (m, 1H, H-7'CH_2), 1.76 (s, 18H, CH_3COOH), 1.59 (q, *J* = 12.6 Hz, 1H, H-2CH_2), 1.07 (d, *J* = 6.1 Hz, 3H, H-9'CH_2); ^{13}C NMR (151 MHz, D_2O) δ 180.56 (C-CH_3*CO*OH), 109.88 (C-1''), 96.29 (C-1'), 95.48 (C-1'''), 84.70 (C-6), 81.55 (C-4''), 77.44 (C-4), 75.32 (C-3''), 73.73 (C-4'), 73.56 (C-2''), 72.63 (C-5), 70.08 (C-5'''), 68.69 (C-8'), 67.55 (C-4'''), 67.26 (C-3'''), 66.88 (C-3'), 66.63 (C-5'), 59.97 (C-5''CH_2), 54.02 (C-2'), 50.77 (C-2'''), 49.87 (C-1), 48.37 (C-3), 46.93 (C-6'), 40.34 (C-6'''CH_2), 34.26 (C-7'CH_2), 29.17 (C-2CH_2), 22.79 (C-CH_3COOH), 19.63 (C-9'CH_3); ESI-HRMS: *m/z* calcd. for $C_{26}H_{51}N_6O_{13}$ $[M+H]^+$ 655.3514, found: 655.3508.

4-O-(2-Amino-5,8-anhydro-2,7,9-trideoxy-D-erythro-α-D-glucononafuranosyl)-5-O-**[3-O-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-2-deoxystreptamine.5AcOH (173):** Following general procedure A, Compound **173** (34.0 mg, 56%) was obtained from **165** (50.0 mg, 0.035 mmol), as a white foam. $[\alpha]_D^{26}$= +63.7 (*c*=0.40, Dichloromethane); ^{1}H NMR (600 MHz, D_2O) δ 5.66 (d, *J* = 5.1 Hz, 1H, H-1'), 5.10 (d, *J* = 1.8 Hz, 1H, H-1'''), 5.05 (d, *J* = 2.5 Hz, 1H, H-1''), 4.46 (t, *J* = 4.7 Hz, 1H, H-3'), 4.30 (t, *J* = 5.7 Hz, 1H, H-3''), 4.28-4.23 (m, 1H, H-6'), 4.17 (dd, *J* = 5.1, 2.5 Hz, 1H, H-6'), 4.14-4.02 (m, 4H, H-4', H-5''', H-8', H-3'''), 4.00 (td, *J* = 6.0, 3.1 Hz, 1H, H-4''), 3.78 (m, 2H, H-2', H-5'), 3.76-3.68 (m, 2H, H-4, H-5''CH_2), 3.62 (br s, 1H, H-4'''), 3.58-3.51 (m, 2H, H-5, H-5''CH_2), 3.46 (t, *J* = 9.8 Hz, 1H, H-6), 3.39 (br s, 1H, H-2'''), 3.31-3.20 (m, 2H, H-3, H-6'''CH_2), 3.19-3.09 (m, 2H, H-1, H-6'''CH_2), 2.28 (dt, *J* = 12.7, 4.4 Hz, 1H, H-2CH_2), 1.82 (dd, *J* = 13.1, 4.5 Hz, 1H, H-7'CH_2), 1.73 (s, 16H, AcOH), 1.64 (q, *J* = 13.5 Hz, 1H, H-2CH_2), 1.60-1.52 (m, 1H, H-7'CH_2), 1.06 (d, *J* = 6.0 Hz, 3H, H-9'CH_3). ^{13}C NMR (151 MHz, D_2O) δ 180.79 (C-CH_3*CO*OH), 110.54 (C-1''), 101.18 (C-1'), 95.33 (C-1'''), 84.65 (C-5), 84.07 (C-5'), 81.29 (C-4''), 79.43 (C-4'), 78.58 (C-4),

75.67 (C-3''), 75.40 (C-8'), 73.44 (C-6'), 73.09 (C-2''), 72.24 (C-3'), 71.81 (C-6'), 70.14, 67.55 (C-5'''), 67.13 (C-4'''), 61.15 (C-5''*CH*$_2$), 58.41 (C-2'), 50.73 (C-2'''), 49.61 (C-1), 48.30 (C-3), 41.42 (C-7'), 40.27 (C-6'''*CH*$_2$), 27.95 (C-2*CH*$_2$), 22.86 (*CH*$_3$COOH), 19.43 (C-9'*CH*$_3$); ESI-HRMS: *m/z* calcd. for $C_{68}H_{74}N_{15}O_{14}Na$ $[M+Na]^+$ 1428.4600, found: 1428.4602.

4-O-(2-Amino-5,8-anhydro-2,7,9-trideoxy-L-threo-β-D-gluco-nonafuranosyl)-5-O-[3-O-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-2-deoxystreptamine.5AcOH (174): Following general procedure A, Compound **174** (14.0 mg, 70%) was synthesized from **166** (30 mg, 0.021 mmol), as a white foam. $[\alpha]_D^{26}$= +60.2 (*c*=0.42, Dichloromethane); ^{1}H NMR (600 MHz, D_2O) δ 5.62 (d, *J* = 5.1 Hz, 1H, H-1'), 5.08 (br s, 1H, H-1'''), 5.04 (d, *J* = 2.5 Hz, 1H, H-1''), 4.44 (t, *J* = 5.0 Hz, 1H, H-3'), 4.33-4.24 (m, 2H, H-6', H-3''), 4.16 (dd, *J* = 5.1, 2.5 Hz, 1H, H-2''), 4.14-4.00 (m, 4H, H-4', H-8', H-3''', H-5'''), 3.99 (td, *J* = 6.3, 3.3 Hz, 1H, H-4''), 3.70 (t, *J* = 5.8 Hz, 1H, H-5'), 3.75 (t, *J* = 4.8 Hz, 1H, H-2'), 3.73-3.66 (m, 2H, H-4, H-5''*CH*$_2$), 3.61 (d, *J* = 3.3 Hz, 1H, H-4'''), 3.56-3.48 (m, 2H, H-5, H-5''*CH*$_2$), 3.43 (t, *J* = 9.9 Hz, 1H, H-6), 3.37 (s, 1H, H-2''), 3.22 (m, 2H, H-1, H-6'''*CH*$_2$), 3.15 (dd, *J* = 13.7, 3.7 Hz, 1H, H-6'''*CH*$_2$), 3.13-3.06 (m, 1H, H-3), 2.31-2.19 (m, 2H, H-7'*CH*$_2$, H-2*CH*$_2$), 1.70 (s, 16H, AcOH),1.65-1.53 (m, 1H, H-2 *CH*$_2$), 1.43 (m, 1H, H-7'*CH*$_2$), 1.07 (d, *J* = 6.2 Hz, 3H, H-9'), ^{13}C NMR (151 MHz, D_2O) δ 181.00 (C-CH$_3$*CO*OH), 110.51 (C-1''), 101.14 (C-1'), 95.31 (C-1'''), 84.66 (C-5), 81.72 (C-5'), 81.22 (C-4''), 79.09 (C-4'), 78.67 (C-4), 75.60 (C-3''), 75.37 (C-8'), 73.25 (C-6'), 73.07 (C-2'''), 72.25 (C-3'), 71.87 (C-6), 70.11 (C-5'''), 67.54 (C-4'''), 67.12 (C-3'''), 61.11 (C-5''), 58.21 (C-2'), 50.71 (C-2''), 49.63 (C-3), 48.27 (C-1), 41.04 (C-7'*CH*$_2$), 40.26 (C-6'''*CH*$_2$), 28.09 (C-2), 23.00 (*CH*$_3$COOH), 20.31 (C-9'*CH*$_3$); ESI-HRMS: *m/z* calcd. for $C_{68}H_{74}N_{15}O_{14}$ $[M+Na]^+$ 1428.4600, found: 1428.4559.

Chapter 4:

General Coupling Protocol: A solution of donor (0.15 mmol), acceptor (0.18 mmol), and activated 4 Å acid-washed powdered molecular sieves (300 mg, 2.0 g/mmol) in anhydrous CH_2Cl_2:MeCN (2:1, 2 mL) was stirred for 0.5 h under Ar, and then cooled to -78 ^oC followed by addition of NIS (42.0 mg, 0.18 mmol) and TfOH (2 μL, 0.02 mmol). The reaction mixture was stirred at -78 ^oC for 5 h and then quenched with DIPEA (7 μL). The mixture was diluted with CH_2Cl_2, filtered through Celite, washed with 20% aqueous $Na_2S_2O_3$ solution, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with EtOAc: hexanes systems to afford the desired coupled products.

Acid washed molecular sieves: 4 Å molecular sieves (30 g) were soaked in 2 *N* HCl (80 mL) for 12 h. The mixture was concentrated under reduced pressure, and then slurried with water (100.0 mL). The slurry was filtered and washed with water (200.0 mL). The resulting solid was dried at 254 ^oC for 24 h to give acid-washed molecular sieves (28.0 g), which were directly used for glycosylation.

General Protocol for Amide Formation from Isothiocyanates[208,209]: To the required 9-fluorenylmethyl (Fm) thioester (0.03 mmol) at room temperature was added a piperidine (0.21 mmol) in DMF (500 μL). The reaction mixture was stirred for 15 min, then diluted with $CHCl_3$ (3.0 mL). The resulting solution was washed with 1*N* HCl aq. (3.0 mL) and brine (3.0 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was dried under high vacuum, and dissolved in dry CH_2Cl_2 (0.5 mL) before addition of the isothiocyanate (0.02 mmol). The reaction mixture was stirred for 36 h at room temperature before the volatiles were removed *in vacuo*. The residue was purified by column chromatography on silica gel eluting with EtOAc: hexanes systems to afford the corresponding amides.

Methyl (1-Adamantanyl 4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-isothiocyanato-2-thio-D-*glycero*-β-D-*galacto*-non-2-ulopyranosid)onate (218): To a stirred solution of **213** (1.5 g, 2.8 mmol) in MeOH (8.0 mL) was added 2M HCl in diethyl ether (8.0 mL) at 0 ºC. The resulting solution was stirred at room temperature for 3.5 h, and then concentrated under reduced pressure. Without further purification the residue was dissolved in MeCN (8.0 mL) and H_2O (16.0 mL), and $NaHCO_3$ (2.3 g, 27 mmol) added. To the vigorously stirred mixture at room temperature was slowly added *O*-phenyl chlorothionoformate (0.8 g, 4.8 mmol) in MeCN (8.0 mL) through a dropping funnel, after which stirring was continued for 1.0 h at room temperature. The resulting mixture was extracted with EtOAc (100 mL x 3), and the combined extracts were washed with brine and then dried over Na_2SO_4 and concentrated. The crude was treated with acetic anhydride (15.0 mL) and pyridine (12.0 mL), stirred at room temperature for 6 h, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with EtOAc/DCM (1/5) to give the desired isothiocyanate **218** (1.0 g, 59%) as a off-white compound with spectral data consistent with those reported in the literature.[180]

Methyl [methyl (4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-isothiocyanato-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→6)-2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (224): Compound **224** was prepared according to general glycosylation procedure using donor **218 (**50.0 mg, 0.08 mmol) and acceptor **219** (43.0 mg, 0.09 mmol) in CH_2Cl_2/CH_3CN (1.2 mL, 2:1) at -78 ºC. After chromatographic purification (gradient elution of EtOAc /Hexanes 2% to 20%) compound **224 (**57.0 mg, 80%) was obtained as a white foam. $[\alpha]^{20}_D$ = -10.3 (*c* =1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.35-7.22 (m, 15H), 5.45 (d, *J* = 9.2 Hz, 1H), 5.33 (m, 1H), 4.94 (d, *J* = 11.7 Hz, 1H), 4.92-4.89 (m, 1H), 4.87 (d, *J* = 11.0 Hz, 1H), 4.75-4.68 (m, 3H), 4.62 (d, *J* = 11.7 Hz, 1H), 4.28-4.26 (m, 2H), 4.15 (dd, *J* = 12.8, 4.4 Hz, 1H), 4.01 (d, *J* = 10.6 Hz, 1H), 3.88-

3.85 (m, 1H), 3.82 (d, *J* = 2.6 Hz, 1H), 3.77 (t, *J* = 9.5 Hz, 1H), 3.63 (s, 3H), 3.58 (t, *J* = 10.3 Hz, 1H), 3.55 (s, 3H), 3.52-3.48 (m, 3H), 2.67 (dd, *J* =13.2, 4.8 Hz, 1H), 2.15 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.05 (s, 3H), 1.75 (t, *J* = 12.5 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 170.7, 169.6, 169.5, 169.4, 167.2 ($^3J_{C\text{-}H}$ = 6.7 Hz), 140.2, 138.8, 138.5, 128.3, 128.2, 128.1, 128.0, 127.6, 127.5, 127.4, 127.3, 104.9, 98.5, 81.9, 79.5, 75.1, 74.2, 73.4, 72.9, 72.6, 71.8, 69.7, 67.9, 67.6, 63.0, 61.7, 57.0, 56.3, 52.9, 37.2, 20.9, 20.8, 20.7, 20.6; ESIHRMS calcd for $C_{47}H_{55}O_{17}NSNa$ ($[M + Na]^+$) 960.3088, found 960.3089.

Methyl [methyl (4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-isothiocyanato-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-2,4-di-*O*-benzyl-β-D-galactopyranoside (225): Compound **225** was prepared according to general glycosylation procedure using donor **218 (**30.0 mg, 0.05 mmol) and acceptor **220** (21.0 mg, 0.06 mmol) in CH_2Cl_2/CH_3CN (0.6 mL, 2:1) at -78 ^{o}C. After chromatographic purification (gradient elution of EtOAc /Hexanes 2% to 20%), compound **225 (**30.0 mg, 79%) was obtained as a white foam. $[\alpha]^{20}_D$ = -11.9 (*c* = 1.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.35-7.27 (m, 10H), 5.43 (d, *J* = 8.8 Hz, 1H), 5.38 (m, 1H), 4.91 (m, 1H), 4.79 (d, *J* = 11.7 Hz, 1H), 4.64 (d, *J* = 11.7 Hz, 1H), 4.58-4.56 (m, 1H), 4.32 (d, *J* = 7.7 Hz, 1H), 4.25 (dd, *J* = 2.2, 12.8 Hz, 1H), 4.04 (dd, *J* = 9.2, 4.7 Hz, 1H), 4.02 (d, *J* = 4.0 Hz, 1H), 3.97 (dd, *J* = 10.6, 1.5 Hz, 1H), 3.80 (s, 3H), 3.78 (d, *J* = 5.9 Hz, 1H), 3.75-3.71 (m, 2H), 3.59 (t, *J* = 5.9 Hz, 1H), 3.55 (m, 4H), 3.51-3.48 (m, 2H), 2.65 (dd, *J* = 13.2, 4.8 Hz, 1H), 2.11 (s, 3H), 2.07 (s, 3H), 2.05 (s, 3H), 1.93 (s, 3H), 1.78 (t, *J* = 12.5 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 170.6, 169.8, 169.3, 169.2, 167.9 ($^3J_{C\text{-}H}$ = 7.5 Hz), 140.3, 138.9, 138.0, 128.36, 128.1, 127.7, 127.4, 104.6, 97.7, 77.3, 75.8, 74.8, 73.5, 72.6, 71.9, 69.7, 69.2, 68.2, 67.9, 67.6, 61.8, 56.9, 56.2, 53.2, 36.3, 29.5, 20.8, 20.7, 20.3; ESIHRMS calcd for $C_{40}H_{49}O_{17}NSNa$ ($[M + Na]^+$) 870.2618, found 870.2619.

Methyl [methyl (4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-isothiocyanato-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (226): Compound **226** was prepared according to general glycosylation procedure using donor **218 (**300.0 mg, 0.5 mmol) and acceptor **221** (260.0 mg, 0.6 mmol) in CH_2Cl_2/CH_3CN (6 mL, 2:1) at -78 °C. After chromatographic purification (gradient elution of EtOAc /Hexanes 2% to 20%), compound **226 (**380 mg, 87%) was obtained as a white foam. $[\alpha]^{20}_D$ = -23.0 (*c* =0.9, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.35-7.22 (m, 15H), 5.43-5.39 (m, 2H), 4.95 (dt, *J* = 10.3, 4.7 Hz, 1H), 4.84 (d, *J* = 11.7 Hz, 1H), 4.80 (d, *J* = 11.4 Hz, 1H), 4.64 (d, *J* = 11.4 Hz, 1H), 4.50 (d, *J* = 11.7 Hz, 1H), 4.44 (d, *J* = 11.4 Hz, 1H), 4.38 (d, *J* = 11.7 Hz, 1H), 4.30-4.26 (m, 2H), 3.99 (dd, *J* = 12.8, 3.7 Hz, 1H), 3.93 (dd, *J* = 9.9, 2.9 Hz, 1H), 3.85 (d, *J* = 10.3 Hz, 1H), 3.74 (s, 3H), 3.68 (d, *J* = 2.6 Hz, 1H), 3.67-3.57 (m, 3H), 3.54 (m, 4H), 3.50 (t, *J* = 10.3 Hz, 1H), 2.58 (dd, *J* = 4.8, 13.6 Hz, 1H), 2.13 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.90 (s, 3H), 1.85 (t, *J* = 12.8 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 170.6, 169.6, 169.3, 169.2, 167.5 ($^3J_{C\text{-}H}$ = 7.0 Hz), 140.3, 139.0, 138.7, 137.9, 128.4, 128.1, 128.0, 127.8, 127.7, 127.6, 127.4, 127.2, 104.9, 98.77, 77.5,76.3, 76.2, 74.8, 73.5, 73.0, 71.6, 69.9, 68.4, 68.0, 67.5, 61.5, 57.0, 56.3, 53.0, 43.3, 35.5, 21.0, 20.8, 20.7, 20.3; ESIHRMS calcd for $C_{47}H_{55}O_{17}NSNa$ ($[M + Na]^+$) 960.3088, found 960.3090.

Benzyl [methyl (4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-isothiocyanato-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-(4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranosyl)-(2→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (227): Compound **227** was prepared according to general glycosylation procedure using donor **218 (**30.0 mg, 0.05 mmol) and acceptor **222** (49.6 mg, 0.06 mmol) in CH_2Cl_2/CH_3CN (0.6 mL, 2:1) at -78 °C. The crude was dissolved in pyridine (1.0 mL) was treated with acetic anhydride (0.8 mL) then stirred overnight at room temperature. The reaction mixture was concentrated under reduced pressure.

After chromatographic purification (gradient elution of EtOAc /Hexanes 2% to 20%) compound **227 (**34.8 mg, 55%) was obtained as a white foam. $[\alpha]^{25}_{D}$ = -21.3 (*c* = 0.3, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.38-7.14 (m, 30H; Ar-H), 5.58 (m, 1H), 5.44 (dd, *J* = 9.2, 1.8 Hz, 1H), 5.06 (d, *J* = 3.3 Hz, 1H), 5.01 (td, *J* = 9.9, 4.4 Hz, 1H), 4.96-4.85 (m, 4H), 4.75-4.67 (m, 3H), 4.62 (dd, *J* = 11.7, 2.7 Hz, 2H), 4.53 (d, *J* = 12.1 Hz, 1H), 4.43 (m, 2H), 4.36 (d, *J* = 11.7 Hz, 1H), 4.31 (dd, *J* = 9.9, 3.6 Hz, 1H), 4.26 (dd, *J* = 12.8, 2.2 Hz, 1H), 4.20 (d, *J* = 12.1 Hz, 1H), 4.07 (dd, *J* = 12.4, 4.0 Hz, 1H), 3.97 (t, *J* = 9.5 Hz, 1H), 3.84 (s, 3H), 3.77 (t, *J* = 9.9 Hz, 1H), 3.71 (dd, *J* = 10.2, 1.8 Hz, 1H), 3.65 (dd, *J* = 11.0, 5.1 Hz, 1H), 3.60 (t, *J* = 6.9 Hz, 1H), 3.52 (m, 2H), 3.43 (m, 2H), 3.29 (m, 3H), 2.67 (dd, *J* = 12.8, 4.7 Hz, 1H), 2.10 (s, 3H), 2.09 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.73 (s, 3H), 1.64 (t, *J* = 12.4 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 170.6, 169.7, 169.6, 169.2, 169.1, 167.2 ($^3J_{C\text{-}H}$ = 6.5 Hz), 140.1, 139.2, 139.1, 138.6, 138.5, 138.0, 137.4, 128.3, 128.2, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.6, 127.6, 127.5, 127.4, 127.3, 127.2, 127.2, 127.1, 127.1, 102.3, 102.0, 97.3, 82.7, 81.8, 79.1, 76.3, 75.0, 74.9, 74.7, 74.0, 73.2, 72.9, 71.5, 70.8, 70.0, 68.7, 68.6, 67.7, 67.5, 67.4, 61.7, 60.0, 56.2, 53.2, 36.8, 21.1, 20.8, 20.7, 20.6, 20.1; ESIHRMS calcd for $C_{75}H_{83}O_{23}NSNa$ ($[M + Na]^+$) 1420.4983, found 1420.4974.

Methyl [methyl (4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-isothiocyanato-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-α(2→9)-[(5-acetamido-2,4,7-tri-*O*-benzoyl-3,5-dideoxy-2-α-D-*glycero*-D-*galacto*-2-nonulopyranose)onate] (228): Compound **228** was prepared according to general glycosylation procedure using donor **218 (**30.0 mg, 0.05 mmol) and acceptor **223** (49.6 mg, 0.06 mmol) in CH_2Cl_2/CH_3CN (0.6 mL, 2:1) at -68 ºC. After chromatographic purification (gradient elution of EtOAc /Hexanes 2% to 20%) compound **228 (**35 mg, 58%) was obtained as a white foam. $[\alpha]^{20}_{D}$ = -10.6 (*c* = 0.5, CH_2Cl_2); ^{1}H NMR (600

MHz, $CDCl_3$) δ: 8.10 (dd, *J* = 7.7, 3.7 Hz, 4H), 7.95 (d, *J* = 7.3 Hz, 2H), 7.63 (t, *J* = 7.3 Hz, 1H), 7.57 (t, *J* = 7.3 Hz, 1H), 7.53-7.44 (m, 5H), 7.37 (t, *J* = 7.7 Hz, 2H), 5.73 (td, *J* = 10.6, 4.7 Hz, 1H), 5.60 (d, *J* = 9.5 Hz, 1H), 5.39 (m, 2H), 5.16 (m, 1H), 4.82 (td, *J* = 9.9, 4.4 Hz, 1H), 4.57 (dd, *J* = 10.6, 1.5 Hz, 1H), 4.26 (m, 2H), 4.10 (dd, *J* = 12.5, 2.2 Hz, 1H), 4.00 (dd, *J* = 10.6, 1.5 Hz, 1H), 3.92 (dd, *J* = 12.8, 2.2 Hz, 1H), 3.83 (s, 3H), 3.68 (m, 4H), 3.46 (m, 2H), 2.88 (dd, *J* = 13.2, 5.1 Hz, 1H), 2.49 (dd, *J* = 13.2, 4.7 Hz, 1H), 2.17 (t, *J* =11.4 Hz, 1H), 2.09 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.84 (s, 3H), 1.57 (t, *J* =12.8 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 170.8, 170.1, 169.8, 169.5, 169.4, 169.3, 167.4 ($^3J_{C\text{-}H}$ = 6.5 Hz), 166.3, 165.4, 164.6, 139.9, 134.0, 133.4, 133.1, 130.0, 130.0, 129.8, 129.2, 128.7, 128.8, 128.8, 98.54, 97.8, 71.9, 69.6, 69.5, 69.5, 68.3, 67.9, 67.8, 66.9, 61.7, 60.0, 56.2, 53.2, 52.9, 49.3, 37.1, 36.7, 29.6, 23.2, 21.0, 20.8, 20.4; ESIHRMS calcd for $C_{52}H_{56}O_{23}N_2SNa$ ($[M + Na]^+$) 1131.2860, found 1131.2892.

Methyl (5-acetamido-2,4,6-tri-*O*-benzoyl-3,5-dideoxy-D-β-*glycero*-D-*galacto*-2-nonulopyranose)onate (223): A stirred solution of methyl ester of *N*-acetylneuraminic acid[210] (1.5 g, 4.64 mmol) in anhydrous dimethylformamide (15.0 mL) was treated with 2,2-dimethoxypropane (1.2 g, 11.5 mmol) and p-toluenesulfonic acid (15.0 mg) at room temperature. The resulting reaction mixture was stirred at 80 ºC for 2 h. The solvent was evaporated under reduced pressure, taken up in pyridine (14 mL) and treated at 0 ºC with benzoyl chloride (3.9 g, 27.7 mmol). After stirring for 1 h at 0 ºC, the volatiles were removed under reduced pressure and the residue was dissolved in dichloromethane (10.0 mL) and was washed with 0.1 N HCl (10.0 mL), saturated aqueous $NaHCO_3$ (2 x 5.0 mL), and brine (5.0 mL). The organic layer was concentrated to afford a yellow form which was treated with TFA (2.3 mL, 80 %) at room temperature for 10 min. The resulted compound was extracted into dichloromethane (10 mL) and

was washed with saturated aqueous $NaHCO_3$ (10.0 mL), water (10.0 mL) and brine (5.0 mL). The organic layer was concentrated to afford a yellow oil that was purified by chromatography on silica gel (EtOAc/Hexanes 5% to 80%) to afford **223**[211] (480.0 mg, 85%) as a white foam. $[\alpha]_D^{26}$= -71 (*c* = 0.4, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 8.10 (m, 4H), 7.95 (d, *J* = 7.3 Hz, 2H), 7.59 (m, 2H), 7.53 (t, *J* = 7.3 Hz, 1H), 7.47 (m, 4H), 7.38 (t, *J* = 7.7 Hz, 2H), 5.64 (m, 2H), 5.27 (d, *J* = 8.8 Hz, 1H), 4.48 (m, 2H), 4.09 (d, *J* = 7.7 Hz, 1H), 3.85 (s, 3H), 3.65 (m, 1H), 3.51 (m, 1H), 2.91 (dd, *J* = 13.2, 4.4 Hz, 1H), 2.28 (t, *J* =11.7 Hz, 1H), 1.79 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 170.1, 166.9, 166.7 ($^3J_{C\text{-}H}$ = 0 Hz), 166.6, 164.9, 129.7, 129.4, 129.0, 128.7, 128.5, 128.4, 98.0, 72.7, 69.4, 69.3, 69.2, 62.5, 53.3, 49.5, 37.0, 23.0; ESIHRMS calcd for $C_{33}H_{33}O_{12}N$ ($[M + Na]^+$) 658.1901, found 658.1888.

Methyl (5-isothiocyanato-4,7,8,9-tetra-*O*-acetyl-2-(dibutylphosphoryl)-3,5-dideoxy-D-glycero-β-D-galacto-non-2-ulopyranoside)onate (231): A solution of thiosialoside donor (300.0 mg, 0.47 mmol), dibutyl phosphate (252.5 mg, 1.20 mmol), and activated 4 Å powdered molecular sieves (100.0 mg, 2.0 g/mmol) in anhydrous CH_2Cl_2 (11.0 mL) was stirred for 1 h under Ar and then cooled to 0 °C followed by addition of NIS (154.8 mg mg, 0.69 mmol) and TfOH (12 μL, 0.14 mmol). The reaction mixture was stirred at 0 °C for 5 h and then quenched with DIPEA (70 μL, 0.47 mmol). The mixture was diluted with CH_2Cl_2 (5.0 mL), filtered through Celite, washed with 20% aqueous $Na_2S_2O_3$ solution, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with EtOAc: toluene (gradient elution 5% to 40%) afforded desired compound (243 mg, 76%) in a 3:2 ratio, as a yellow oil.

α-isomer: $[\alpha]_D^{26}$= −22.4 (*c*=1.45, CH_2Cl_2); ^{1}H NMR (600 MHz, Chloroform-*d*) δ 5.44 (dd, *J* = 9.2, 1.4 Hz, 1H), 5.23 (ddd, *J* = 9.1, 4.1, 2.4 Hz, 1H), 4.97 (ddd, *J* = 12.1, 9.9, 4.7 Hz,

1H), 4.29 (dd, *J* = 12.6, 2.4 Hz, 1H), 4.19 (dd, *J* = 10.6, 1.4 Hz, 1H), 4.15 (dd, *J* = 12.7, 4.1 Hz, 1H), 4.09-4.03 (m, 1H), 4.03-3.96 (m, 3H), 3.80 (s, 3H), 3.63 (t, *J* = 10.3 Hz, 1H), 2.73 (dd, *J* = 13.0, 4.8 Hz, 1H), 2.29 (t, *J* = 12.6 Hz, 1H), 2.12 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 1.61 (m, 4H), 1.36 (m, 4H), 0.89 (td, *J* = 7.4, 5.4 Hz, 6H); ^{13}C NMR (151 MHz, CH_2Cl_2) δ 170.62, 169.53, 169.49, 169.43, 166.51 ($^3J_{C\text{-}H}$ = 7.2 Hz), 140.66, 97.91, 97.86, 77.26, 77.05, 76.83, 72.74, 70.02, 69.36, 68.95, 68.25, 68.04, 68.00, 67.90, 67.86, 67.22, 61.34, 56.82, 56.10, 53.35, 36.81, 36.78, 32.06, 32.03, 32.01, 31.98, 20.92, 20.83, 20.71, 20.56, 18.57, 18.53, 13.54, 13.51; **^{31}P NMR** (151 MHz, Chloroform-*d*) δ -6.96; ESI-HRMS: *m/z* calcd. for $C_{27}H_{42}NO_{15}$ PSNa $[M+Na]^+$ 706.1911, found: 706.1893.

β-isomer: $[\alpha]_D^{26}$= −45.8 (*c*=0.95, CH_2Cl_2); ^{1}H NMR (600 MHz, Chloroform-*d*) δ 5.50 (dd, *J* = 6.5, 1.8 Hz, 1H), 5.36 (ddd, *J* = 11.4, 9.9, 4.9 Hz, 1H), 5.23 (td, *J* = 6.1, 2.4 Hz, 1H), 4.43 (dd, *J* = 12.5, 2.5 Hz, 1H), 4.29 (dd, *J* = 10.6, 1.8 Hz, 1H), 4.23 (dd, *J* = 12.6, 5.7 Hz, 1H), 4.12-4.02 (m, 5H), 3.80 (s, 3H), 3.68 (t, *J* = 10.3 Hz, 1H), 2.72 (dd, *J* = 13.7, 4.9 Hz, 1H), 2.15 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 1.90 (ddd, *J* = 14.1, 11.4, 3.2 Hz, 1H), 1.64 (m, 4H), 1.38 (m, 4H), 0.92 (td, *J* = 7.4, 3.8 Hz, 6H); ^{13}C NMR (151 MHz, Chloroform-*d*) δ 170.60, 169.91, 169.44, 169.31, 165.63 ($^3J_{C\text{-}H}$ = 0 Hz), 140.74, 98.90, 71.82, 69.80, 68.84, 68.49, 68.45, 68.40, 68.27, 61.77, 56.31, 53.29, 36.64, 36.61, 32.11, 32.06, 29.67, 20.90, 20.87, 20.76, 20.63, 18.58, 13.54; **^{31}P NMR** (151 MHz, Chloroform-*d*) δ −6.17; ESI-HRMS: *m/z* calcd. for $C_{27}H_{42}NO_{15}$ PSNa $[M+Na]^+$ 706.1911, found: 706.1887.

General protocol for glycosylation with sialyl phosphate donor 231: A solution of donor **231** (0.05 mmol), acceptor (0.06 mmol), and activated 4 Å powdered molecular sieves (100 mg, 2.0 g/mmol) in anhydrous CH_2Cl_2 (1.0 mL) was stirred for 1 h under Ar, and then cooled to -78 ^{o}C followed by addition of TMSOTf (0.07 mmol). The reaction mixture was stirred

at -78 ºC for 6-8 h and then quenched with TEA (0.05 mmol). The mixture was diluted with CH_2Cl_2, molecular sieves were filtered off and reaction mixture was washed with brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give crude reaction mixtures which were purified by chromatography over silica gel using EtOAc:hexanes systems to afford the desired coupled products.

Competition reaction:

A solution of isothiocyanate donor **208** (42.0 mg, 0.06 mmol), *N*-acetyl-5-*N*,4-*O*-oxazolidinone-protected adamantanyl thiosialoside donor 2**06**[173] (41.0 mg, 0.06 mmol), acceptor **221** (30.4 mg, 0.06 mmol) and activated 4 Å acid-washed powdered molecular sieves (150.0 mg) in anhydrous CH_2Cl_2:MeCN (2:1, 0.5 mL) was stirred for 0.5 h under Ar, and then cooled to -78 ºC followed by addition of NIS (7.0 mg, 0.06 mmol) and TfOH (1.0 μL, 0.01 mmol). The reaction mixture was stirred at -78 ºC for 5 h and then quenched with DIPEA (10.0 μL). The mixture was diluted with CH_2Cl_2, filtered through Celite, washed with 20% aqueous $Na_2S_2O_3$ solution, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel eluting with EtOAc: hexanes systems to afford the coupled products, **230 (**31.0 mg, 51%)[173] and compound **226** (2.0 mg, 3%) and the unreacted donors **218** (31.0 mg, 74%) and **206** (7.0 mg, 17%).

Methyl [methyl (4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy--*α*-D-*gluco*-2-nonulopyranosyl)onate]-(2→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (236): To a solution of **226** (50.0 mg, 0.05 mmol) in anhydrous toluene (1.5 mL) under Ar was added tris(trimethylsilylsilane) (65.0 mg, 0.26 mmol) followed by azoisobutyronitrile (1.0 mg, 0.006 mmol) at room temperature. The resulting reaction mixture was stirred at 85 ºC for 1 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column

chromatography eluting with 30% EtOAc in hexanes to give the title compound **236** (36.0 mg, 78%) as a yellow foam. $[\alpha]_D^{25}$= -7.4 (*c* = 2.3, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.39 (d, *J* = 6.9 Hz, 2H), 7.34-7.20 (m, 13H), 5.44 (m, 1H), 5.17 (dd, *J* = 8.4, 2.2 Hz, 1H), 4.91 (d, *J* = 11.7 Hz, 1H), 4.82 (m, 2H), 4.71 (d, *J* = 11.3 Hz , 1H), 4.52 (d, *J* = 11.7 Hz, 1H), 4.48 (d, *J* = 11.3 Hz, 1H), 4.41 (d, *J* = 11.7 Hz, 1H), 4.32 (d, *J* = 7.7 Hz, 1H), 4.29 (dd, *J* = 12.8, 2.5 Hz, 1H), 4.05 (m, 2H), 3.94 (dt, *J* = 12.1, 1.8 Hz, 1H), 3.73 (d, *J* = 2.5 Hz, 1H), 3.68 (s, 3H), 3.67-3.57 (m, 4H), 3.54 (s, 3H), 2.50 (dd, *J* = 11.7, 4.4 Hz, 1H), 2.10 (s, 3H), 2.02 (s, 3H), 1.97 (s, 3H), 1.92 (s, 3H), 1.89 (m, 1H), 1.80 (t, *J* = 12.4 Hz, 1H), 1.24 (m, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 170.4, 169.9, 169.8, 169.6, 168.3, 139.2, 138.9, 138.1, 128.3, 128.0, 127.9, 127.8, 127.7, 127.2, 127.0, 104.9, 99.5, 77.6, 76.6, 75.8, 74.8, 73.4, 73.2, 70.6, 69.4, 68.8, 68.4, 67.1, 61.7, 60.0, 57.0, 52.6, 36.1, 32.0, 21.0, 20.9, 20.6, 20.4; ESIHRMS calcd for $C_{46}H_{56}O_{17}Na$ ($[M + Na]^+$) 903.3415, found 903.3432.

Methyl [methyl (4,7,8,9-tetra-*O*-acetyl-5-*C*-allyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-4-*O*-acetyl-2,6-di-*O*-benzyl-β-D-galactopyranoside (238): Acetic anhydride (1.0 mL) added to a solution of **225** (60.0 mg, 0.07 mmol) in pyridine (1.5 mL) at 0 ºC and the resulting reaction mixture stirred for 4 h. The reaction mixture was concentrated under reduced pressure and was purified by chromatography to give a pentaacetate which was taken forward to the next step without further characterization. To a solution of this pentaacetate **237** (62.0 mg) in anhydrous benzene (1.0 mL) under Ar was added allyltris(trimethylsilylsilane) (502.0 mg, 1.74 mmol) followed by azoisobutyronitrile (11.4 mg, 0.04 mmol) at room temperature. The resulting reaction mixture was stirred at 80 ºC for 12 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography eluting with 30% EtOAc in hexanes to give the title compound **238** (27.0 mg,

45%) as yellow foam. $[\alpha]_D^{25}$= -2.2 (*c*= 0.7, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$) δ: 7.41 (d, *J* = 7.7 Hz, 2H), 7.32-7.20 (m, 8H), 5.62 (m, 1H), 5.57 (m, 1H), 5.42 (d, *J* = 8.4 Hz, 1H), 5.05 (d, *J* = 3.3 Hz, 1H), 5.02 (m, 2H), 4.86 (td, *J* = 11.4, 4.7 Hz, 1H), 4.80 (m, 2H), 4.52 (d, *J* = 11.7 Hz, 1H), 4.45 (d, *J* = 12.1 Hz, 1H), 4.44 (d, *J* = 8.4 Hz, 1H), 4.38 (dd, *J* = 9.4, 2.9 Hz, 1H), 4.30 (dd, *J* = 12.4, 1.8 Hz, 1H), 4.10 (dd, *J* = 12.8, 4.0 Hz, 1H), 3.80 (m, 3H), 3.67 (d, *J* = 11.0 Hz, 1H), 3.56 (s, 3H), 3.53-3.41 (m, 4H), 2.57 (dd, *J* = 12.1, 4.4 Hz, 1H), 2.22 (dd, *J* = 14.3, 3.6 Hz, 1H), 2.10 (s, 3H), 2.08 (m, 1H), 2.03 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.76 (s, 3H), 1.59 (t, *J* = 12.1 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 170.6, 170.0, 170.0, 169.8, 169.7, 168.2, 139.6, 138.0, 133.2, 128.2, 127.9, 127.6, 127.5, 127.1, 126.9, 117.7, 104.5, 97.0, 78.1, 74.3, 73.4, 72.7, 72.0, 72.0, 69.3, 69.2, 68.7, 68.5, 68.0, 62.1, 57.3, 52.7, 38.9, 37.6, 30.3, 21.2, 20.9, 20.8, 20.7, 20.3; ESIHRMS calcd for $C_{44}H_{56}O_{18}Na$ ($[M + Na]^+$) 895.3364, found 895.3358.

***S*-(9-Fluorenylmethyl) benzyloxythioacetate (242):** To a ~2.0 M solution of 2-(benzyloxy)acetic acid (60.0 mg, 0.36 mmol), 9-fluorenylmethylthiol[209] (100.0 mg, 0.47 mmol) and DMAP (5.0 mg, 0.04 mmol) in CH_2Cl_2 (1.0 mL), was added a solution of DCC (82.0 mg, 0.39 mmol) in CH_2Cl_2 (0.4 mL) at 0 ºC**.** The suspension was stirred for 1 h at 0 ºC and overnight at room temperature. The suspension was filtered to remove the resulting white solid which was washed with CH_2Cl_2 (2.0 mL**)** repeatedly. The filtrate was concentrated and purified by chromatography over silica gel to give the title thioester (242) as a colorless oil (128.0 mg, 98%). $\alpha]_D^{26}$= +30.0 (*c* = 1.2, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$) δ: 7.76 (d, *J* = 7.7 Hz, 2H), 7.69 (d, *J* = 7.3 Hz, 2H), 7.42 (t, *J* = 7.3 Hz, 2H), 7.39-7.29 (m, 7H), 4.48 (s, 2H), 4.22 (t, *J* = 5.9 Hz, 1H), 4.10 (s, 2H), 3.59 (d, *J* = 5.9 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 199.7, 145.4, 141.2, 136.8, 128.5, 128.1, 128.1, 127.7, 127.2, 124.7, 119.9, 74.7, 73.7, 46.7, 31.1; ESIHRMS calcd for $C_{23}H_{20}O_2SNa$ ($[M + Na]^+$) 383.1082, found 383.1073.

Methyl [methyl (4,7,8,9-tri-*O*-acetyl-5-(benzyloxyacetamido)-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (246): Compound **246** was prepared according to general protocol for amide formation using isothiocyanate **221 (**0.03 g, 0.05 mmol) and Fm thioester **242** (49.6 mg, 0.06 mmol) in CH_2Cl_2 (0.5 mL) at 40 ^oC. After chromatographic purification (gradient elution of EtOAc/Hexanes 4% to 40%) compound **246 (**20.0 mg, 55%) was obtained as a white foam. $[\alpha]^{20}_D$ = -5.3 (*c* = 0.3, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$) δ: 7.40-7.19 (m, 20H), 6.31 (d, *J* = 10.3 Hz, 1H), 5.47 (m, 1H), 5.29 (dd, *J* = 8.8, 2.2 Hz, 1H), 4.89 (dt, *J* = 11.7, 4.7 Hz, 1H), 4.86 (d, *J* = 11.7 Hz, 1H), 4.82 (d, *J* = 12.1 Hz, 1H), 4.72 (d, *J* = 12.1 Hz, 1H), 4.58 (d, *J* = 11.7 Hz, 1H), 4.53 (d, *J* = 11.7 Hz, 1H), 4.49 (d, *J* = 11.7 Hz, 2H), 4.42 (d, *J* = 11.7 Hz, 1H), 4.34 (d, *J* = 7.3 Hz, 1H), 4.28 (dd, *J* = 12.4, 2.5 Hz, 1H), 4.10 (m, 2H), 3.95 (dd, *J* = 12.4, 4.7 Hz, 1H), 3.92-3.81 (m, 4H), 3.70 (s, 3H), 3.68-3.59 (m, 4H), 3.53 (s, 3H), 2.52 (dd, *J* = 13.1, 4.7 Hz, 1H), 2.12 (s, 3H), 2.05 (t, *J* = 13.1, 1H), 1.98 (s, 3H), 1.95 (s, 3H), 1.92 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 170.4, 170.3, 170.2, 169.8, 169.7, 168.2, 139.1, 138.1, 136.7, 128.8-126.9, 104.8, 98.8, 77.6, 76.3, 76.2, 74.8, 74.6, 73.5, 73.4, 73.0, 72.1, 69.1, 68.6, 68.5, 67.0, 62.0, 57.1, 52.8, 48.4, 36.7, 29.6, 21.1, 20.7, 20.7, 20.6; ESIHRMS calcd for $C_{55}H_{65}NO_{19}Na$ ($[M + Na]^+$) 1066.4049, found 1066.4042.

Methyl [methyl (4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-5-(L-methioninamido)-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (247): Compound **247** was prepared according to general amide formation procedure using isothiocyanate **221 (**30.0 mg, 0.02 mmol) and 9-fluorenylmethyl thioester of *N*-*tert*-Butoxycarbonyl-L-methionine **245**[209] (12.0 mg, 0.06 mmol) in CH_2Cl_2 (0.5 mL) at 40 ^oC. After chromatographic purification (gradient elution of EtOAc /Hexanes 10% to 90%) compound **247 (**18 mg, 50%) was obtained as a white foam. $[\alpha]^{25}_D$ = -13.8 (*c* = 0.5, CH_2Cl_2); ^1H

NMR (600 MHz, $CDCl_3$) δ: 7.38 (d, *J* = 7.3 Hz, 2H), 7.34-7.19 (m, 15H), 6.10 (d, *J* = 9.5 Hz, 1H), 5.46 (m, 1H), 5.17 (d, *J* = 8.4 Hz, 1H), 5.06 (d, *J* = 5.8 Hz, 1H), 4.90-4.84 (m, 2H), 4.82 (d, *J* = 11.7 Hz, 1H), 4.71 (d, *J* = 11.7 Hz, 1H), 4.48 (d, *J* = 11.7 Hz, 2H), 4.42 (d, *J* = 11.7 Hz, 1H), 4.34 (d, *J* = 7.3 Hz, 1H), 4.22 (d, *J* = 12.1 Hz, 1H), 4.14 (q, *J* = 7.3 Hz, 1H), 4.08 (dd, *J* = 9.9, 2.5 Hz, 1H), 4.04-3.94 (m, 2H), 3.90 (d, *J* = 7.3 Hz, 1H), 3.73 (d, *J* = 11.3 Hz, 1H), 3.72 (s, 3H), 3.70-3.63 (m, 2H), 3.64-3.58 (m, 3H), 3.53 (s, 3H), 2.53 (dd, *J* = 13.2, 4.7 Hz, 1H), 2.10 (s, 6H), 2.03 (t, *J* = 8.4 Hz, 1H), 2.00 (s, 3H), 1.96 (s, 3H), 1.90 (s, 3H), 1.80 (m, 1H), 1.47 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 171.7, 170.4, 170.2, 169.9, 169.6, 168.1, 156.1, 139.2, 138.1, 128.3, 128.0, 127.9, 127.7, 127.6, 127.6, 127.6, 127.1, 127.0, 104.9, 98.5, 80.5, 77.63, 76.3, 76.2, 74.8, 74.6, 73.4, 73.0, 72.1, 68.8, 68.6, 68.4, 67.2, 62.1, 60.0, 57.0, 52.7, 53.6, 48.8, 36.5, 30.1, 29.6, 28.3, 21.0, 20.8, 20.7, 20.6, 15.0; ESIHRMS calcd for $C_{56}H_{74}O_{20}N_2SNa$ ($[M + Na]^+$) 1149.4453, found 1149.4462.

Methyl [methyl (4,7,8,9-tetra-*O*-acetyl-5-(benzyloxyacetamido)-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosyl)onate]-α(2→9)-[5-acetamido-8-*O*-acetyl-2,4,7-tri-*O*-benzoyl-3-deoxy-2-α-D-*glycero*-D-*galacto*-nonulopyranosel)onate] (249): Acetic anhydride (0.8 mL) added to a solution of compound **228** (24.0 g, 0.02 mmol) in pyridine (1.0 mL) at 0 ºC and the resulting reaction mixture stirred for 4 h. The reaction mixture was concentrated under reduced pressure and was purified by chromatography to give a pentaacetate which was taken forward to the next step without further characterization. Compound **249** was prepared according to general amide formation procedure using isothiocyanate **248** (24.0 mg) and Fm thioester **242** (12.0 mg, 0.06 mmol) in CH_2Cl_2 (0.5 mL) at 40 °C. After chromatographic purification (gradient elution of EtOAc/Hexanes 10% to 90%), compound **249 (**12.0 mg, 46%) was obtained as a white foam. $[\alpha]^{25}_D$ = -13.3 (*c* = 0.6, CH_2Cl_2); ^1H NMR (600 MHz, $CDCl_3$) δ: 8.15 (d, *J* = 7.7 Hz, 2H),

8.13 (d, *J* = 7.7 Hz, 2H), 7.95 (d, *J* = 7.7 Hz, 2H), 7.62 (t, *J* = 7.3 Hz, 1H), 7.58 (t, *J* = 7.7 Hz, 1H), 7.54-7.45 (m, 5H), 7.40-7.30 (m, 7H), 6.22 (d, *J* = 10.3 Hz, 1H), 5.97 (dt, *J* = 10.6, 4.7 Hz, 1H), 5.78 (d, *J* = 8.8 Hz, 1H), 5.66 (d, *J* = 8.4 Hz, 1H), 5.32 (m, 1H), 5.21 (dd, d, *J* = 8.4, 1.8 Hz, 1H), 4.93 (m, 1H), 4.75 (dt, *J* = 10.2, 4.4 Hz, 1H), 4.64 (d, *J* = 10.6 Hz, 1H), 4.55 (d, *J* = 11.7 Hz, 1H), 4.50 (d, *J* = 11.7 Hz, 1H), 4.06-3.96 (m, 2H), 3.93-3.85 (m, 4H), 3.84-3.80 (m, 5H), 3.71 (s, 3H), 3.67 (dd, *J* = 12.8, 4.7 Hz, 1H), 3.57 (dd, *J* = 11.3, 2.9 Hz, 1H), 2.91 (dd, *J* = 13.5, 5.1 Hz, 1H), 2.46 (dd, *J* = 13.2, 4.7 Hz, 1H), 2.18 (t, *J* = 13.2 Hz, 1H), 2.07 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.93 (s, 3H), 1.84 (t, *J* =12.8 Hz, 1H), 1.71 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 170.6, 170.3, 170.3, 170.1, 169.7, 169.7, 169.1, 167.8, 166.6, 166.0, 165.4, 163.5, 136.7, 133.8, 133.3, 130.1, 130.0, 129.7, 129.3, 129.3, 128.7, 128.6, 128.5, 128.4, 128.2, 128.0, 98.3, 97.8, 73.4, 72.2, 70.9, 69.6, 69.1, 68.9, 68.5, 67.9, 67.8, 66.6, 62.4, 61.7, 60.0, 53.0, 52.7, 50.8, 48.2, 37.5, 36.5, 23.5, 21.0, 20.7, 20.7, 20.4; ESIHRMS calcd for $C_{62}H_{68}N_2O_{26}Na$ ($[M + Na]^+$) 1279.3928, found 1279.3939.

Methyl [methyl (4,7,8,9-tetra-*O*-acetyl-5-(*N*'-(2-phenylethyl)thioureido)-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-[2,4,6-tri-*O*-benzyl-β-D-galactopyranoside] (250): To a solution of isothiocyanate **226** (65.0 mg, 0.06 mmol) in anhydrous DCM (1.8 mL) under Ar was added 2-phenylethylamine (10.0 mg, 0.8 mmol) at room temperature. The resulting reaction mixture was stirred at room temperature for 1 h, then quenched with 1N HCl and washed with water (2.0 mL), and brine (2.0 mL). The solvent was evaporated under reduced pressure to give the title compound **250** (72.0 mg, 90%) as yellow foam. $[\alpha]_D^{25}$= -9.4 (*c* = 0.4, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.40 (d, *J* = 7.3 Hz, 3H), 7.36-7.19 (m, 17H), 6.24 (br s, 1H), 5.40 (td, *J* = 7.7, 2.2 Hz, 1H), 5.27 (m, 1H), 4.95 (br s, 1H), 4.89 (d, *J* = 11.3 Hz, 1H), 4.84 (d, *J* = 12.1 Hz, 1H), 4.72 (d, *J* = 11.7 Hz, 1H), 4.50 (t, *J* = 11.7

Hz, 2H), 4.43 (d, *J* = 11.7 Hz, 2H), 4.34 (d, *J* = 7.7 Hz, 1H), 4.12 (d, *J* = 8.8 Hz, 1H), 3.98 (dd, *J* = 12.4, 6.2 Hz 1H), 3.89 (m, 1H), 3.70 (d, *J* = 2.2 Hz, 1H), 3.68 (s, 3H), 3.67-3.59 (m, 4H), 3.54 (s, 3H), 2.85 (m, 2H), 2.46 (dd, *J* = 13.2, 4.0 Hz, 1H), 2.14 (m, 1H), 2.09 (s, 3H), 1.96 (s, 9H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 171.2, 170.4, 170.4, 170.1, 169.9, 168.2, 139.2, 138.1, 128.7, 128.3, 127.9, 127.8, 127.7, 127.6, 127.6, 127.1, 127.1, 126.6, 104.6, 98.9, 77.5, 76.4, 76.2, 74.8, 74.6, 73.4, 73.0, 72.5, 70.0, 69.6, 68.6, 67.5, 62.1, 60.0, 57.1, 54.5, 52.7, 36.0, 34.9, 21.3, 21.1, 20.9, 20.7; ESIHRMS calcd for $C_{55}H_{66}O_{17}N_2SNa$ ($[M + Na]^+$) 1081.3938, found 1081.3980.

Methyl [5-(*N*'-(2-phenylethyl)guanidino)-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulo-pyranosylonate]-(2→3)-[2,4,6-tri-*O*-benzyl-β-D-galactopyranoside] (252): To a solution of thiourea **250** (83.0 mg, 0.07 mmol) in anhydrous dichloromethane (2.0 mL) under Ar was added DMAP (1.0 mg, 0.008 mmol) followed by DIPEA (50.0 mg, 0.38 mmol) at room temperature. The resulting reaction mixture was treated with methyl iodide (33.0 mg, 0.23 mmol) drop wise and stirred at room temperature for 30 h, then quenched with 0.1N HCl (2.0 mL) and was washed with water (2.0 mL), and brine (2.0 mL). The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography eluting with 30% EtOAc in hexanes to give the isothiourea **251**[212] (57.0 mg, 80%) as a colorless liquid, which was taken forward to the next step without further characterization. A stirred solution of compound **251** (33 mg, 0.03 mmol) in anhydrous dimethylformamide (1.5 mL) was transferred into a glass tube and cooled to -33 °C. Dry gaseous ammonia was then passed into the reaction for 5min ,after which the reaction mixture was stirred at 0 ºC for 5 min to enable evaporation of excess ammonia. The glass tube was sealed and the reaction was stirred at 130 ºC for 36 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel chromatography (eluent: gradient elution of 5-60% ammonical methanol in EtOAc) to give the

title compound **252** (11.0 mg, 49%) as a colorless oil. $[\alpha]_D^{25}$= -10.1 (*c*=0.7, dichloromethane); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.46 (d, *J* = 7.3 Hz, 3H), 7.36-7.16 (m, 17H), 5.00 (d, *J* = 11.3 Hz, 1H), 4.84 (d, *J* = 11.3 Hz, 1H), 4.75 (d, *J* = 11.3 Hz, 1H), 4.60 (br s, 1H), 4.56 (d, *J* = 11.7 Hz, 1H), 4.39 (d, *J* = 11.7 Hz, 1H), 4.35-4.29 (m, 2H), 4.25 (d, *J* = 7.7 Hz, 1H), 3.96 (d, *J* = 2.2 Hz, 1H), 3.93 (m, 1H), 3.78 (dd, *J* = 11.3, 2.5 Hz, 2H), 3.65-3.60 (m, 2H), 3.60-3.54 (m, 2H), 3.54 (t, *J* = 7.7 Hz, 1H), 3.49 (m, 4H), 3.42 (m, 1H), 3.39-3.34 (m, 2H), 3.30 (m, 1H), 2.89 (dd, *J* = 12.4, 4.7 Hz, 1H), 2.81 (m, 2H), 1.79 (t, *J* = 12.1 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 173.3, 157.4, 139.1, 138.6, 137.9, 137.8, 128.4, 128.2, 128.2, 128.0, 127.9, 127.8, 127.7, 127.5, 127.2, 127.1, 127.0, 126.32, 104.5, 100.1, 78.1, 75.8, 75.1, 74.9, 74.7, 73.2, 72.8, 72.6, 71.8, 69.1, 68.9, 62.8, 60.0, 55.9, 48.0, 42.8, 40.5, 34.5; ESIHRMS calcd for $C_{46}H_{58}O_{13}N_3$ ($[M + H]^+$) 860.3970, found 860.3945.

Methyl [Sodium (3,5-dideoxy-α-D-*gluco*-2-nonulopyranosyl)onate]-(2→3)-[β-D-galactopyranoside] (253): Sodium methoxide (3.0 mg, 0.05 mmol) was added to a solution of compound **236** (26.0 mg, 0.03 mmol) in MeOH (1.0 mL). After stirring for 1 h at room temperature, the mixture was diluted with MeOH (1.0 mL) and 2N NaOH (0.2 mL) and refluxed at 70 ºC, for 2 h. The solution was neutralized with Amberlyst-15, filtered through small plug of Celite, and the filter plug washed with MeOH (2.0 mL). The combined filtrates were concentrated under reduced pressure to furnish a residue, which was taken up in phosphate buffer (*p*H =7, 1.0 mL) treated with 5% Pd on carbon (30.0 mg), and stirred at room temperature for 15 h under 1 atm of H_2. The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo. The residue was purified by chromatography on a Sephadex G-10 column (eluent: water) and then by chromatography on Dowex 50 WX8-100 sodium ion exchanger eluting with water. The resulting solution was frozen in a dry-ice/acetone bath, and then

lyophilized to give compound **253** (11.5 mg, 91%) as a white foam. $[\alpha]^{20}_{D}$ = -12.3 (*c* = 0.7, H_2O); ^{1}H NMR (600 MHz, D_2O) *δ*: 4.17 (d, *J* = 8.1 Hz, 1H), 3.86 (dd, *J* = 9.5, 2.9 Hz, 1H), 3.72 (d, *J* = 2.9 Hz, 1H), 3.69-3.63 (m, 3H), 3.61 (d, *J* = 12.1 Hz, 1H), 3.57-3.50 (m, 2H), 3.48-3.41 (m, 2H), 3.37 (br s, 1H), 3.36 (s, 3H), 3.31 (t, *J* = 9.5 Hz, 1H), 2.46 (dd, *J* = 11.7, 3.6 Hz, 1H), 1.62 (d, *J* = 9.9 Hz, 1H), 1.33 (t, *J* = 11.7 Hz, 1H), 1.30 (d, *J* = 12.1 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 174.3, 103.4, 100.0, 75.4, 74.8, 72.1, 71.2, 71.0, 69.0, 67.4, 65.3, 62.3, 60.8, 56.8, 40.2, 33.9; ESIHRMS calcd for $C_{16}H_{27}O_{13}$ ($[M - H]^-$) 427.1452, found 427.1461.

Methyl [sodium (3,5-dideoxy-5-*C*-propyp-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-β-D-galactopyranoside (254): Sodium methoxide (1.7 mg, 0.03 mmol) was added to a solution of compound **238** (14.0 mg, 0.02 mmol) in MeOH (0.5 mL). After 30 min of stirring at room temperature, the mixture was diluted with MeOH (1.0 mL) and 2N NaOH (0.2 mL) and refluxed at 70 ºC, for 2 h. The solution was neutralized with amberlyst-15 and filtered through small plug of celite and was washed with MeOH (2.0 mL). The solution was then concentrated under reduced pressure to furnish a crude residue. The solution of the deacetylated product in phosphate buffer (*p*H =7, 0.5 mL) with suspended 5% Pd on carbon (17.0 mg) was stirred at room temperature for 8 h under H_2 atmosphere. The whole mixture was filtered through celite, and the filtrate was concentrated in vacuo. The residue was purified through a Sephadex G-10 column and passed through Dowex 50 WX8-100 sodium ion exchanger both using water as eluent. The resulting solution was frozen in a dry-ice/acetone bath, and then lyophilized to get compound **254** (7.0 mg, 93%) as a white foam. $[\alpha]^{20}_{D}$ = -18.2 (*c* = 0.5, H_2O); ^{1}H NMR (600 MHz, D_2O) *δ*: 4.19 (d, *J* = 8.1 Hz, 1H), 3.89 (dd, *J* = 9.9, 3.3 Hz, 1H), 3.73 (d, *J* = 2.9 Hz, 1H), 3.72-3.68 (m, 2H), 3.55 (m, 4H), 3.49 (dd, *J* = 8.4, 4.0 Hz, 2H), 3.47-3.42 (m, 2H), 3.38 (s, 3H), 3.33 (t, *J* = 8.1 Hz, 1H), 2.47 (dd, *J* = 12.1, 4.4 Hz, 1H), 1.43 (t,

J = 11.7 Hz, 1H), 1.31 (m, 1H), 1.24 (m, 1H), 1.16 (m, 1H), 1.01 (m, 1H), 0.69 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 174.5, 103.4, 99.4, 75.3, 74.8, 73.7, 72.3, 69.0, 68.4, 67.8, 67.3, 62.5, 60.8, 60.0, 56.8, 41.4 , 27.3 , 17.6 , 13.9; ESIHRMS calcd for $C_{19}H_{33}O_{13}$ ($[M - H]^-$) 469.1921, found 469.1907.

Methyl [sodium (3-dideoxy-5-(glyceramido)-D-*glycero*-α-D-*galacto*-2-nonulopyranosyl)onate]-(2→3)-β-D-galactopyranoside (255): Sodium methoxide (1.6 mg, 0.02 mmol) was added to a solution of compound **242** (16.0 mg, 0.015 mmol) in MeOH (0.5 mL). After 30 min of stirring at room temperature, the mixture was diluted with MeOH (1.0 mL) and 2N NaOH (0.2 mL) and refluxed at 70 ºC, for 2 h. The solution was neutralized with amberlyst-15 and filtered through small plug of celite and was washed with MeOH (2.0 mL). The solution was then concentrated under reduced pressure to furnish a crude residue. The solution of the deacetylated product in phosphate buffer (*p*H =7, 0.5 mL) with suspended 5% Pd on carbon (30.0 mg) was stirred at room temperature for 20 h under H_2 atmosphere. The whole mixture was filtered through celite, and the filtrate was concentrated in vacuo. The residue was purified through a Sephadex G-10 column and passed through a Dowex 50 WX8-100 sodium ion exchanger both using water as eluent. The resulting solution was frozen in a dry-ice/acetone bath, and then lyophilized to get compound **255** (7.0 mg, 91%) as a white foam. $[\alpha]^{25}_D$ = +0.4 (*c* = 0.5, H_2O); ^{1}H NMR (600 MHz, D_2O) *δ*: 4.18 (d, *J* = 8.1 Hz, 1H), 3.91 (s, 2H), 3.89 (dd, *J* = 9.9, 3.3 Hz, 1H), 3.74 (d, *J* = 2.9 Hz, 1H), 3.72 (d, *J* = 10.2 Hz, 1H), 3.67 (m, 2H), 3.59 (dd, *J* = 11.3, 4.4 Hz, 1H), 3.63-3.50 (m, 3H), 3.47 (m, 1H), 3.43 (dd, 1H), 3.38 (d, *J* = 9.2 Hz, 1H), 3.36 (s, 3H), 3.33 (t, *J* = 8.1 Hz, 1H), 2.57 (dd, *J* = 12.1, 4.4 Hz, 1H), 1.60 (t, *J* = 12.1 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 175.6, 173.7, 103.8, 99.6, 75.7, 74.8, 72.4, 71.7, 69.0, 67.9, 67.8,

67.3, 62.3, 60.8, 60.8, 56.8, 51.2, 39.5; ESIHRMS calcd for $C_{18}H_{30}O_{15}N$ ([M - H]$^-$) 500.1615, found 500.1633.

Methyl [sodium (3-dideoxy-5-(*N*'-(2-phenylethyl)guanidino)-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate]-(2→3)-β-D-galactopyranoside (256): The solution of **252** (15.0 mg, 0.02 mmol) in methanol (1.0 mL) was treated with 5% Pd on carbon (30.0 mg) was stirred at room temperature for 10 h under 1 atm of H_2. The mixture was filtered through Celite, and the filtrate was concentrated in vacuo. The residue was purified through a Sephadex C-25 column and passed through a Dowex 50 WX8-100 sodium ion exchanger both using water as eluent. The resulting solution was frozen in a dry-ice/acetone bath, and then lyophilized to get compound **256** (5.2 mg, 52%) as white foam. $[\alpha]^{20}_D$ = -10.7 (*c* = 0.1, H_2O); ^{1}H NMR (600 MHz, D_2O) δ: 7.21 (t, *J* = 7.3 Hz, 2H), 7.13 (m, 3H), 4.19 (d, *J* = 7.7 Hz, 1H), 3.89 (dd, *J* = 9.9, 2.9 Hz, 1H), 3.76-3.70 (m, 2H), 3.68 (dd, *J* = 12.1, 2.5 Hz, 1H), 3.60-3.51 (m, 3H), 3.50-3.43 (m, 4H), 3.38 (s, 3H), 3.37-3.30 (m, 3H), 3.21 (t, *J* = 9.2 Hz, 1H), 2.72 (dt, *J* = 6.6, 1.8 Hz, 2H), 2.57 (dd, *J* = 12.4, 4.7 Hz, 1H), 1.55 (t, *J* = 12.1 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 173.4, 156.4, 138.4, 128.8, 128.7, 126.9, 103.4, 99.4, 75.6, 74.7, 72.5, 71.9, 69.0, 68.4, 68.1, 67.1, 62.3, 60.8, 56.9, 54.3 , 42.6, 39.8, 34.2; ESIHRMS calcd for $C_{25}H_{40}O_{13}N_3$ ([M - H]$^-$) 588.2405, found 588.2422.

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ABSTRACT

SYNTHESIS OF APRAMYCIN AND PAROMOMYCIN DERIVATIVES AS POTENTIAL NEXT GENERATION AMINOGLYCOSIDE ANTIBIOTICS AND CHEMISTRY OF ISOTHIOCYANATO SIALYL DONORS

by

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AGAs are clinically important antibacterials for human therapy and have long been used as highly potent antibiotics for treating several bacterial infections. The fidelity of protein synthesis is affected by AGAs in the course of binding to specific sites of the bacterial rRNA. The clinical use of AGAs and their applications as therapeutics is restricted by toxicity (irreversible ototoxicity and reversible nephrotoxicity) and by the resistance of pathogens. The objective of this research was the development of proficient AGAs that are less toxic (*i.e.*, more selective) and that evade resistance. The first three chapters of this thesis are aimed towards developing new aminoglycoside antibiotics with the emphasis on their chemical synthesis, and the biological evaluation of newly synthesized analogues, as well as the exploration of structure-activity relationships to understand the mechanism of their antimicrobial activity. In particular, studies have focused on the modification of the aminoglycosides apramycin and paromomycin so as to develop the next generation of potent AGAs.

Chapter two reveals the importance of the 6' and *N*7' positions of the apramycin by investigation of the antibacterial activity and antiribosomal activity of the ten apramycin derivatives which were synthesized by modifying these locations. The effect of such

modifications on antiribosomal activity is discussed in terms of their influence on drug binding to specific residues in the decoding A site. This information is useful in the development of a structure activity relationship for the antibacterial activity of the apramycin class of aminoglycosides and will also assist in the future design and development of more active and less toxic aminoglycoside antibiotics.

Chapter three describes the structure-based design of an improved paromomycin derivative which carries an apramycin-like bicyclic ring I and a conformationally restricted hydroxyl or amine functionality. The influence of the bicyclic paromomycin 6'-hydroxy or amine groups on the binding pattern between AGA and bacterial RNA was investigated by using cell free translational assays. It was found that the bicyclic paromomycin derivative **155** with the equatorial 6'-hydroxy group has a better activity profile than parent paromomycin.

In chapter four, an efficient sialyl donor was developed for the challenging α-sialylation by means of a highly electron withdrawing isothiocyanato group incorporated at C-5 position sialic acid. The isothiocyanato sialyl donor **218** proved to be an excellent α-directing group in sialylation for a wide range of acceptors, and provided high yields. Further, the sialylation of corresponding sialyl phosphate donor **231** was also demonstrated to give excellent selectivity, but yields are lower due to competing elimination. In addition, the rich chemistry of isothiocyanate functionality is explored to introduce a variety of novel functionalities at the 5-position of the sialosides including deamination, an alkyl chain, various amides, and guanidine derivatives.

AUTOBIOGRAPHICAL STATEMENT

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- Appi Reddy Mandhapati; Andrea Vasella; Erik C. Böttger; David Crich. "Structure-Based Design and Synthesis of Novel Apramycin Paromomycin Analogues. Importance of the Configuration at the 6'-Position and Differences Between the 6'-Amino and Hydroxy Series" Manuscript in preparation.
- Appi Reddy Mandhapati; Takayuki Kato; Takahiko Matsushita; Bashar Ksebati; Andrea Vasella; Erik C. Böttger; David Crich, *J. Org. Chem*. **2015**, *80* (3), 1754–1763.
- Appi Reddy Mandhapati; Salla Rajender; Jonathan Shaw; David Crich, *Angew. Chem. Int. Ed.* **2015**, *54*, 1275 –1278 (designated as a Hot Paper).
- Appi Reddy Mandhapati; Dimitri Shcherbakov; Stefan Duscha; Andrea Vasella; Erik C. Böttger; David Crich, *ChemMedChem* **2014**, *9*, 2074-2083 (designated as a Very Important Paper).

POSTER PRESENTATION

- Presented a poster at 250th ACS National Meeting in Boston, Massachusetts held in August 16-20, 2015. Title: Influence of the isothiocyanato moiety on stereoselective synthesis of sialic acid glycosides and subsequent diversification.